

# **Examining the use of coral sand for the treatment of domestic effluent in Kiribati**

Bronwyn Louise Humphries

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# Abbreviations

AGW	Artificial Groundwater
ADB	Asian Development Bank
cfu	colony forming units
DNA	Deoxyribonucleic acid
EC	Electrical Conductivity
E. faecalis	Enterococci faecalis
E. coli J6-2	Escherichia coli J6-2
g	gram
LRV	Log Removal Value
MDG	Millennium Development Goal
mm	millimeters
MS2 phage	F-RNA MS2 bacteriophage
ND	Not Detected
PSD	Particle Size Distribution
PBS	Phosphate Buffered Saline
pfu	plaque forming units
PV	Pore Volume
RNA	Ribonucleic acid

SEM	Scanning Electron Microscope
SDG	Sustainable Development Goal
TSB	Tryptic Soy Broth
UV	Ultraviolet
UN	United Nations
WASH	Water, Sanitation, Hygiene
WHO	World Health Organization
ZP	Zeta Potential

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# Abstract

Laboratory based, unsaturated drainage experiments were undertaken using worked Bikenibeu beach coral sand from South Tarawa in Kiribati, to examine its drainage and effective microbial removal properties. Bacterial and viral indicators (*E. coli* J6-2, *E. faecalis* and MS2 phage) along with viral pathogens (adenovirus; echovirus; norovirus; rotavirus) were drained under gravity through coral sand-packed columns, serving as physical models of a domestic effluent drainage field. Experiments using clean coral sand and coral sand which were conditioned with Dissolved Organic Carbon (DOC), in the form of domestic effluent, were investigated. The results show that coral sand has a higher affinity for attenuating viruses than bacteria. All organisms examined showed removal efficiencies over 4-Log Removal Values (LRVs) which is equivalent to a 99.99 % reduction in the target microorganisms.

Attenuation mechanisms such as absorption and to a lesser extent physical straining likely play a major role in the ability of coral sand to attenuate the microbial tracers used in this study. The application of DOC in the form of domestic effluent may have provided additional binding sites on the surface of the coral sand and increased the adsorption rate of viruses but the bacterial indicators (*E. coli* J6-2, *E. faecalis*) did not appear to be influenced by DOC. It was also found that changes in ionic strength, such as rainfall, increased the mobilisation of microorganisms within coral sand. Long term field scale studies are required to verify the laboratory results as well as incorporating the effects of kind tide events, fluctuations in groundwater, effects of prolonged rainfall and examining microbial transport within coral sand under saturated conditions.

These findings could have important implications for the use of locally available materials, such as coral sand, to improve household onsite wastewater treatment in Kiribati and offer enhanced protection of groundwater resources and reduce diarrheal disease.

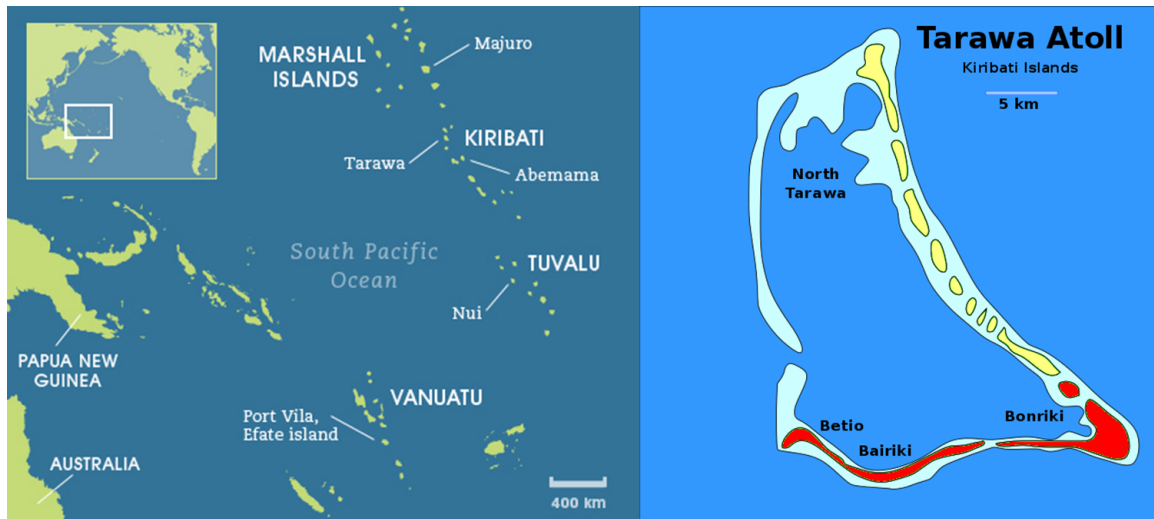


# 1. Introduction

## 1.1. Statement of the problem

Sewage disposal is a critical sanitation issue affecting Pacific atoll communities [Storey and Hunter, 2010, White and Falkland, 2010]. One of the most acute examples is the Republic of Kiribati (Figure 1.1), where the burden of pathogenic disease due to poor sanitation is significant [RNZ, 2014, Tabunga et al., 2014]. There are strong links between inadequate Water, Sanitation and Hygiene (WASH), and the incidence of diarrheal disease, with Kiribati having one of the highest rates of infant mortality in the Pacific region [ADB, 2014]. A 2013 survey of Kiribati households found that the incidence of WASH related diseases reached an estimated annual incidence of 50,000 – 55,000 cases or approximately 50% of the population [ADB, 2014]. Significant diarrheal outbreaks of viral pathogens such as rotavirus have been recorded in Kiribati since 2010 and average about three per year [RNZ, 2014, Tabunga et al., 2014, WHO, 2016]. These diarrheal disease outbreaks tend to occur during national celebrations or public holidays and are aggravated by large gatherings of people coupled with inadequate sanitation facilities [ADB, 2014, WHO, 2016].

South Tarawa is the capital and most populated atoll of Kiribati (Figure 1.1). The population density of South Tarawa is just over 4,900 people per km<sup>2</sup> which almost equals the population density of London. With the population increasing at an extraordinary rate (72% by 2050), the impact of inadequate sanitation is acute. Its sanitation practices and infrastructure, however, have not developed in proportion to population growth. The majority of the population on South Tarawa have traditionally practiced open defecation on the beach or in the lagoon [WHO, 2016]. Only a very small proportion of the atoll is connected to the reticulated ocean outfall sewage system, and the only alternative to open defecation is the use of unlined pit latrines or basic septic tanks. The biggest problem with unlined pit

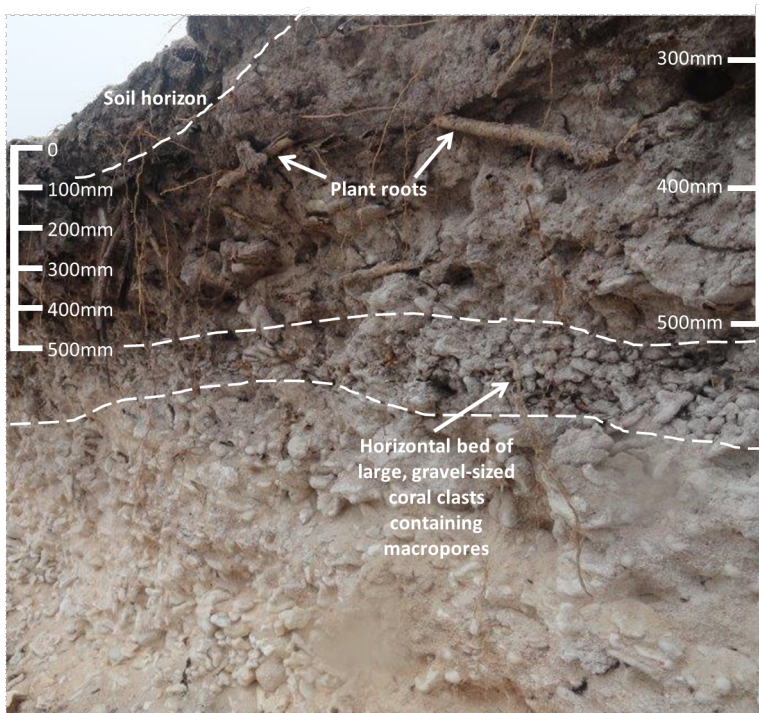


**Figure 1.1.:** Location map of the Republic of Kiribati (left) and Tarawa atoll (right), with South Tarawa in red and North Tarawa in yellow. Image sources: [www.commonswikimedia.org](http://www.commonswikimedia.org) and [www.scidev.net](http://www.scidev.net) respectively.

latrines and septic tanks is the microbial contamination risk to the shallow freshwater lenses which are approximately 1 - 2 metres below ground level and are used for drinking water [White et al., 2007, Werner et al., 2017]. These freshwater lenses are the only resource of freshwater on South Tarawa. Pit latrines and septic tanks act as conduits to directly contaminate the freshwater lens with faecal waste. In addition, contamination of the vulnerable freshwater lenses are exacerbated by the presence of preferential pathways caused by highly permeable horizontally layered beds of large, gravel-sized coral clasts which are not supported by finer deposits as shown in Figure 1.2 [P. Sinclair, personal communication, February 19, 2018]. These beds have the ability to transport contaminants quickly over long distances.

In an on-going effort to improve sanitation practices on atolls, such as South Tarawa, alternative modes of sewage disposal are being explored. However, the limited resources of many atolls restrict the available options, and require that any engineered wastewater disposal system should be affordable, sustainable and utilise technology which is locally accessible and easily maintained. Septic tank systems are one of the options being assessed. In South Tarawa, examples can be found where septic tank systems have recently been installed to treat waste from households fitted with flush toilets. Expectations are that more septic tank systems will be installed in the future, as the population and demand for access to

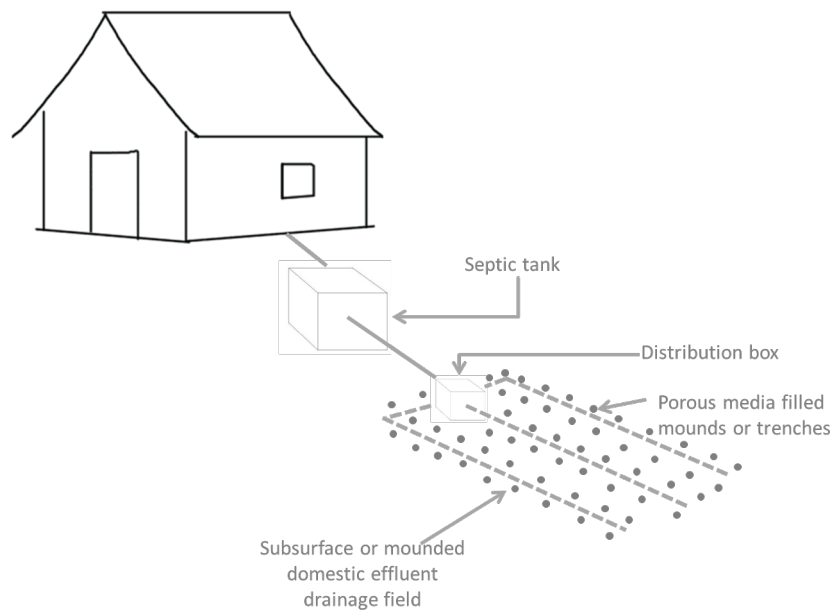




**Figure 1.2.:** A profile of highly permeable horizontally layered beds of large, gravel-sized coral clasts on the ocean side of South Tarawa, Kiribati.

improved sanitation grows. It is assumed that the majority of septic tank systems on South Tarawa do not incorporate any secondary stage treatment. More importantly, a significant shortcoming in their installation design lies in their lack of a designed septic tank effluent drainage field (Figure 1.3), over which liquid waste can be discharged and where a level of treatment can occur. This is a key component of any septic tank system to enhance microbial removal. In most cases, discharge from the septic tank is via a point discharge that directly enters the surrounding (undisturbed) atoll substrate. As previously mentioned, this is a hazard to the shallow freshwater lenses and is exacerbated by preferential flow pathways. It can be assumed that the pathogen loads to the subsurface environment from these septic tank systems is significant. Aside from contamination of the freshwater lenses an additional health risk lies with exposure to ponded effluent that can occur at the point of wastewater discharge during periods of heavy rainfall. It is proposed that a properly designed and engineered septic tank effluent drainage field could conceivably contribute to improving inadequate sanitation on South Tarawa. Burbery et al. [2016] investigated the ability of coral sand sourced from South Tarawa to attenuate model pathogens

within disturbed laboratory column experiments. Their results indicated that coral sand has a greater attenuation for *Escherchia coli* (*E. coli*) in comparison to viruses. Using graded coral sand in a septic tank effluent drainage field conceivably would limit the risk of preferential flow, thereby mitigating contamination of the freshwater lenses from faecal waste. It is important to note however that there is no evidence supporting the ability of coral sand to attenuate nutrients.



**Figure 1.3.:** Example septic tank and land application of effluent via a drainage field (not to scale).

## 1.2. Research aims and objectives

The aim of this research was to investigate the effective properties and performance of coral sand as a porous granular filter medium, as might be used in an engineered septic tank effluent drainage field. The specific research question was: How effective is coral sand at attenuating selected microorganisms, as related to domestic effluent? The research was executed through practical microbial tracer column experiments made on unsaturated coral sand. The objectives were:

1. Evaluate effective pathogen removal rates on: i) clean coral sand, ii) coral sand conditioned with domestic effluent

2. Study the impact of a flush of groundwater and a high rainfall event
3. Examine the packing properties of coral sand and the risk of preferential flow
4. Examine the microbial attenuation properties of coral sand

### **1.3. Organisation of thesis**

This thesis comprises six chapters. After the introduction in Chapter 1, Chapter 2 provides a review of the scientific literature related to this research topic. This includes the impact of inadequate sanitation globally, the Pacific region and Kiribati, alongside a geographical description of Kiribati and the physical properties of coral sands that are found there. Mechanisms for microbial removal in granular porous media are described along with the characteristics of model pathogenic microorganisms typically used in microbial tracing experiments. A literature review description of studies most relevant to the ability of Kiribati coral sand to remove microorganisms from onsite effluent treatment systems is included in the review.

The three chapters that follow provide the methods, results and discussion of the physiochemical properties of coral sand (Chapter 3); the clean coral sand experiments (Chapter 4) and the conditioned coral sand experiments (Chapter 5). The final chapter (Chapter 6) lists the main conclusions from the research, and provides recommendations for future research on the potential use of coral sand as a domestic effluent disposal field material.



## **2. Literature review**

### **2.1. The issue of inadequate sanitation**

#### **2.1.1. Global perspective**

Provision of adequate and equitable sanitation is a critical global issue and a daunting task [Pruss-Ustun, 2014, Roche et al., 2017, WHO and UNICEF, 2017]. The challenge to increase global sanitation coverage was set under the Millennium Development Goals (MDGs, 1990 - 2105) by the United Nations (UN) and has now transferred to the Sustainable Development Goals (SDGs, 2016-2030). At the end of the MDGs in 2015, more than 70% of the world's population (5.3 billion) still lacked access to safely managed sanitation facilities [WHO and UNICEF, 2017]. In the same year, 892 million people worldwide continued to practice open defecation [WHO and UNICEF, 2017]. The SDG goal to reach everyone everywhere with sanitation, without leaving anyone behind (Goal 6), is immense [WHO and UNICEF, 2017].

Inadequate access to sanitation and water supply cost the global economy US\$260 billion in 2012 [WHO, 2012] and more than US\$200 billion in 2015 [LIXEL, 2016]. These costs are associated with socio-economic issues, poor health and environmental impacts. The impact of inadequate sanitation has a significant effect on the health and well-being of the population, economy and environment of developing countries in particular [WHO-UNEP, 2004, Hutton and Chase, 2016]. The inappropriate disposal of human waste has a detrimental impact on ecosystems, marine and freshwater resources [Templeton, 2015]. People who rely on unsafe freshwater resources (that are contaminated by the inappropriate disposal of human waste) for drinking, are impacted greatly by diarrhoeal disease [WHO and UNICEF, 2013] with diarrhoeal disease being the most significant consequence of inadequate WASH, causing 842,000 deaths in 2012

[Roche et al., 2017]. The impact of diarrhoeal disease is most acute in children under the age of five and is the leading cause of child mortality worldwide [Roche et al., 2017].

There are many contributing factors as to the lack of sanitation progress globally, particularly in developing countries. It is well documented that urban sanitation globally has progressed at a much faster rate than rural sanitation [Templeton, 2015, WHO and UNICEF, 2017, Massoud et al., 2009]. The inequalities between rural and urban sanitation are strongly linked to wealth [LIXEL, 2016, Roche et al., 2017]. There are also challenging issues around the implementation of reliable and affordable sanitation options in developing countries [Massoud et al., 2009]. Furthermore, changing imbedded unsafe sanitation behaviour is difficult when influenced by sanitation beliefs, attitudes, normally accepted behaviour, convenience and cost [Mosler, 2012].

### **2.1.2. Pacific region**

Strongly linked to inadequate WASH practices, diarrhoeal disease claims the lives of over 1,000 people annually in the Pacific region [WHO, 2016]. The infrastructure cost of achieving universal coverage of adequate sanitation for the Pacific region by 2030 is estimated to be US\$80 million annually [WHO, 2016]. The Pacific also contains some of the most vulnerable countries in the world facing the effects of climate change [WHO, 2016]. Increasing climate variability, extreme weather events and natural hazards make meeting international targets for improving sanitation coverage within the Pacific region an immense challenge.

Tracking towards improving sanitation coverage in the Pacific region during the MDG period (1990 – 2015) stagnated with only a 2% gain achieved [WHO and UNICEF, 2015]. With over 80% of the total Pacific population living in rural areas, the Pacific is replicating a global trend in that rural areas lag behind urban areas in terms of sanitation coverage [WHO and UNICEF, 2015] (United Nations, 2014). During the MDG period, the Pacific experienced a population growth of 70% indicating that developments in sanitation have not kept pace with population growth [WHO, 2016]. High population growth and the low priority given to sanitation investments over the last 20 years are two of the main reasons for the lack of progress on improving sanitation in the Pacific [WHO, 2016].

Approximately 70% of the total estimated 10 million people living in the Pacific do not currently have access to improved sanitation [WHO, 2016]. The number of people in the Pacific region still practicing open defecation equals the world average of 13% [WHO, 2016]. In addition to open defecation, sanitation options commonly include decentralised systems such as pit latrines and poorly functioning septic tanks. Centralised infrastructure for wastewater collection, treatment and disposal in the Pacific is uncommon and only limited to those middle income countries such as Rarotonga and Fiji and centred around urban areas [Dakers and Evans, 2007].

Similar to global findings, the Pacific region's health and well-being, economy and environment are significantly impacted by inadequate sanitation [Bower et al., 2002]. The freshwater lenses of small islands and low lying atolls within the Pacific are particularly vulnerable to contamination from human effluent [WHO, 2016, Dillion, 1997]. Pacific islands with a greater topographic relief, such as islands of volcanic origin, generally have a greater capability to attenuate contaminants sourced from human effluent due to thicker unsaturated zones, higher clay and organic matter contents in the soils [Dillion, 1997]. The impact on groundwater quality due to inadequate sanitation is particularly severe on small islands and atolls where reticulated water supplies and centralised sewage collection and treatment are generally unavailable [Dillion, 1997]. Many Pacific island village households have their own sanitation system which is either a pit latrine, or a poorly designed septic tank, or they rely on open defecation as a means of waste disposal. For all of these sanitation options there is an obvious risk to human health where the underlying shallow groundwater lens is used as a source of fresh drinking water [Dillion, 1997]. Within a tropical environment any benefits of the thermal inactivation of pathogenic microorganisms are most likely overwhelmed by moist humid conditions enhancing survival and migration of pathogens within groundwater coupled with shallow groundwater systems which have a high hydraulic connection to surface land use [Dillion, 1997]. Calcareous sediments that make up the substrate of atolls are very heterogeneous. The presence of preferential flow paths due to larger coarse coral fragments can result in the rapid transport of contaminants over long distances, further contributing to the contamination of freshwater lenses [Dillion, 1997].

## 2.2. The Republic of Kiribati

### 2.2.1. Country profile

Within the Pacific region South Tarawa, Kiribati, faces one of the most challenging sanitation situations. This is due to challenges on multiple fronts such as population growth, lack of infrastructure, vulnerable environment, and limited resources which all impact on the provision of adequate sanitation and the appropriate disposal of human effluent [ADB, 2014].

The Republic of Kiribati includes 33 low-lying atolls straddling the equator half way between Fiji to the south and Hawaii to the north, located in Micronesia [Falkland and Woodroffe, 1997] (Figure 1.1). The atolls are divided into three groups: Gilbert, Phoenix and Line Islands Groups. The Exclusive Economic Zone of Kiribati is the second largest in the Pacific totalling an area of approximately 3.5 million km<sup>2</sup> while the total land area only slightly exceeds 800 km<sup>2</sup> [Thomas, 2003].

The Kiribati atolls developed from volcanos between 55 - 65 million years ago during the Paleocene geological epoch [Stanley, 2001]. Over time, the peaks of the volcanos sunk below sea level and the atolls were formed by successive coral deposits surrounding the submerged volcanos [Stanley, 2001]. Kiribati atolls generally consist of an internal lagoon which occupies the site of the former volcano core [ADB, 2011]. The maximum land height above sea level for these atolls is typically 1 – 2 meters [Falkland and Woodroffe, 1997].

Tarawa atoll is triangular in shape, as shown in Figure 1.1, with a shallow lagoon and is included in the Gilbert Island Group [Falkland and Woodroffe, 1997, Paulay and Kerr, 2001]. It is split into two parts, North Tarawa and South Tarawa with each containing a string of islets. South Tarawa is the political and economic capital of Kiribati and extends from the islets of Bonriki in the south to Betio in the west with these islets connected by a series of artificial causeways [Falkland and Woodroffe, 1997]. North Tarawa is much less populated and consists of traditional rural communities which stretch from the islet of Buota in the south to Buariki in the north and are not connected by causeways [Falkland and Woodroffe, 1997]. As a whole, Tarawa atoll consists of a series of islets that are rarely more than 100 – 400 meters wide [Woodroffe and Biribo, 2011].



South Tarawa is one of the most densely populated locations in the Pacific (approximately 5,500 people per km<sup>2</sup>), with more than half the country's total population of 110,000 thousand people residing on a useable land area of 10 km<sup>2</sup> [GoK, 2016, Tabunga et al., 2014]. In 2016 the population of South Tarawa was 56,388, and is expected to double by 2030 [ADB, 2014, GoK, 2016]. The remaining population of Kiribati reside on the other 32 atolls, known as the Outer Islands. These atolls are considered traditional rural communities. They contain different reef and lagoon configurations but are essentially the same geological composition as Tarawa atoll. The Outer Islands are less developed than South Tarawa and are constrained by isolation, transport, low-incomes, limited natural and imported resources alongside limited education, health, and infrastructure.

Alongside king tide events, heavy rainfall events are becoming more frequent on South Tarawa as the climate changes [Burton et al., 2011]. These heavy rainfall events provide challenges for onsite domestic effluent disposal systems in that they present the opportunity for systems to become saturated potentially resulting in the rapid transport of faecal contaminants within the freshwater lenses.

### 2.2.2. Sanitation status

The status of sanitation in South Tarawa differs from the Outer Islands of Kiribati, mainly due to differences in population and infrastructure. Sanitation infrastructure and water supply on South Tarawa has been described by the Asian Development Bank as 'being in a dilapidated state' [ADB, 2014]. Centralised sewerage systems are available to the residents of only three settlements; Bairiki, Betio, and Bikenibeu. Bairiki and Betio are the Government and commercial centres, respectively [Woodroffe and Biribo, 2011]. The sewerage is discharged via ocean outfalls which are located on the ocean side of each of the above three settlements. The residents on South Tarawa not connected to the centralised sewerage system practice open defecation or rely on pit latrines and basic poorly designed septic tank systems with no effluent disposal field. Similar to global practices, these septic tanks are often intentionally designed to leak with the perception that they will never need to be emptied [WHO and UNICEF, 2017]. Composting toilets on South Tarawa are an emerging and developing sanitation technology but progress is still needed on community acceptance due to the taboo around the handling of human faeces [RNZ, 2013].

The status of sanitation on the Outer Islands is similar to that of South Tarawa with the exception of the settlements on South Tarawa that are connected to the three ocean outfalls as mentioned above. Initiatives such as the Community Total-Led Sanitation movement are attempting to end the practice of open defecation in the Outer Islands [Kar, 2005]. Pit latrines on the Outer Islands are common but basic poorly designed septic tank systems with no effluent disposal field are becoming more frequent. Like South Tarawa, composting toilets on the Outer Islands are a new sanitation technology with community acceptance developing [RNZ, 2013].

### **2.2.3. The impact of inadequate sanitation**

Inadequate sanitation has had a significant negative impact on the densely populated South Tarawa but has also affected the less populated Outer Islands of Kiribati. Similar to global and Pacific regional trends, as discussed in Section 2.1.1 and Section 2.1.2, poor access to sanitation impacts the health, economy and environment of Kiribati [ADB, 2014]. The economic cost of inadequate sanitation and water supplies, has been calculated by the Asian Development Bank [ADB, 2014] for South Tarawa. The report includes both market and non-market costs in the form of health expenditure, loss in economic productivity, reduced benefits from tourism and environmental pollution [ADB, 2014]. Conservative estimates calculate that the annual economic burden of poor sanitation (including water) on South Tarawa is \$3.7 – 7.3 million [ADB, 2014]. This equates to an annual economic cost of US\$500 – 1,000 per household in South Tarawa [ADB, 2014].

Alongside the economic cost of inadequate sanitation in Kiribati the environmental impact is significant as well. The atolls of Kiribati are recent weathered reef deposits and contain freshwater lenses which are the only source of potable water [Falkland and Woodroffe, 1997]. There is anecdotal evidence from observations of exposed coral substrate deposits near the ocean side of South Tarawa to suggest that macroporous pathways exist within the substrate which provide conduits for contaminants to be rapidly transported within the freshwater lenses [P. Sinclair, personal communication, February 19, 2018]. As previously mention in Section 1.1 these highly porous preferential flow pathways, consisting of macroporous deposits, exist within the atoll substrate and are caused by layered larger coral clasts and shell fragments which are not supported by finer coral sand deposits. These macroporous deposits are a significant issue when the water table is typically 1 - 2

metres below ground level and in some instances it has been found that surface contaminants can reach the water table in less than 1 hour [Werner et al., 2017, White et al., 2006]. Septic tanks and pit latrines which intersect the water table and the presence of macroporous layers provide the opportunity for the freshwater lenses to be contaminated by faecal waste. Faecal matter from open defecation can also leach into the underlying freshwater lenses contributing to the contamination. It is widely accepted on South Tarawa that large areas of the fresh groundwater lenses are not suitable for drinking water due to the significant faecal contamination [White and Falkland, 2010]. In summary, nearly all sanitation systems on South Tarawa contribute to the pollution of the freshwater lenses and surrounding coastal and lagoon waters.

The cost of inadequate sanitation within Kiribati has been most heavily carried by the population's health. Second only to Papua New Guinea, Kiribati has the worst child mortality rate for under 5 years in the Pacific region with water-related diseases, such as diarrhoeal disease, being significant causes [WHO, 2016]. With an estimated annual incidence of 50,000 – 55,000 cases of WASH related diseases the health impact on the population of Kiribati is significant [ADB, 2014]. Outbreaks of viral pathogens, such as rotavirus, have been recorded in Kiribati since 2010 and average about three per year [Tabunga et al., 2014, WHO, 2016].

### 2.2.4. The future of sanitation in Kiribati

WHO [2016] states that there are a few basic principles that should orientate the sanitation sector in the Pacific region as a whole. The principles are especially applicable to Kiribati in the years to come:

- Sanitation solutions need to be sustainable, safe, and not adversely impact fragile water resources
- More needs to be done to empower small, isolated and informal communities to safely and sustainably manage their own sanitation
- A significant increase in support is required to strengthen the capacity of Pacific island governments, utilities and communities to manage sustainably sanitation services in the face of human and financial constraints.

- Safe and sustainable sanitation solutions are vital to strengthen and maintain the resilience of Pacific communities to the increasing threats of climate variability, climate change and natural hazards.

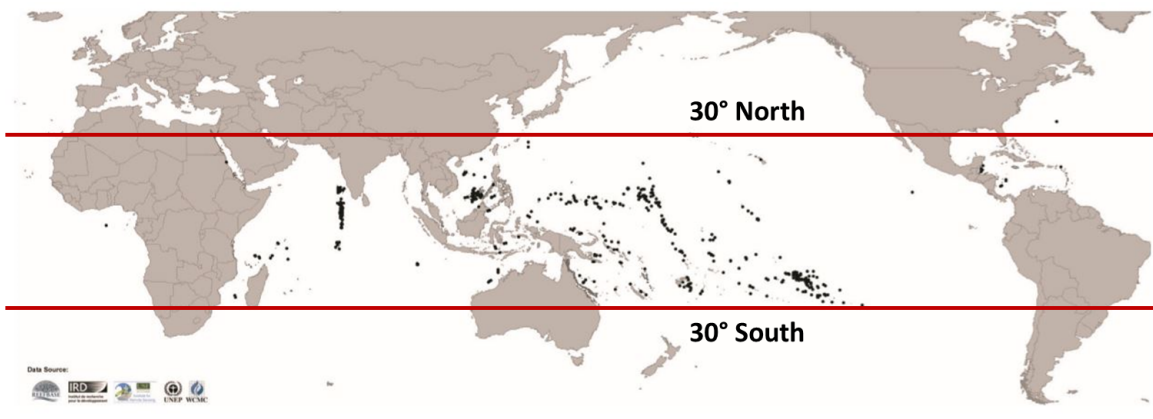
WHO [2016] recommends that in order to meet defined health related targets of the SDGs, Kiribati will have to improve sanitation outcomes at a significantly faster rate than has been achieved in the past. Accelerated progress will most likely hinge on the development of sustainable innovative sanitation technologies that utilise locally available materials that are within the reach of not only the population on South Tarawa but also the Outer Islands of Kiribati [WHO, 2016].

Pfannes et al. [2015] states that various pathogen reducing wastewater treatment technologies are currently available but that the uptake of these technologies is often hampered by a countries economic situation. Wastewater treatment technologies therefore should be simple, low-cost and utilise local materials [Pfannes et al., 2015]. One natural resource that both South Tarawa and the Outer Islands have is coral sand. As with other porous natural materials, coral sand may be able to be used as a granular filter to treat domestic effluent but remains largely unexplored.

## **2.3. Coral sediments**

### **2.3.1. Geographic distribution**

Globally, coral sand (also referred to as carbonate or calcareous sand) are found between the latitudes of 30° N and 30° S as shown in Figure 2.1 [UNEP/IUCN, 1988, Mallik, 1999]. Coral sands are found within the vicinity of coral reefs which are categorised into either shelf reefs, which form on continental shelves of large land masses; or oceanic reefs, which form next to deep ocean waters on islands or atolls. Coral reefs and their by-product coral sand, are abundant in warm (in excess of 22°C), clear, shallow ocean waters of the Caribbean, Hawaii, Indonesia, Indian Ocean, Australian north coast, Red Sea and the Pacific [Stanley, 2001, Morrison, 1990]. As Figure 2.1 shows, coral sand can be found amongst the 425 atolls of the world with most of these found in the Pacific and Indian Oceans (Falkland, 1991; Falkland, 1992).



**Figure 2.1.:** The global location of atolls, as an indication of the distribution of coral sand globally, marked as black dots between the latitudes 30 North and 30 South. Image source: <http://www.reefbase.org>

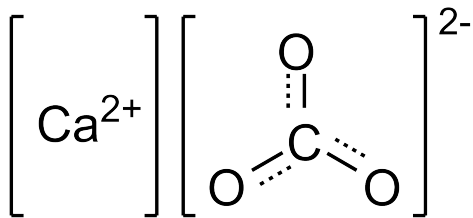
### 2.3.2. Origins

Coral sand is created upon the death of protists, plants and animals which leave behind their calcareous shells and skeletons [Stanley, 2001]. Carbonate sediments are found on continental shelves, oceanic banks and atolls, and nearshore environments where the terrestrial inputs are few [Stanley, 2001]. Coral sands found on atolls, which is the focus of this research, originate from the coral reef systems surrounding atolls [Wild et al., 2006]. Once physical erosion processes and bio-erosion occur the coral sands that are produced form the permeable unconsolidated Holocene coral sand deposits that overlie permeable Pleistocene limestone reef deposits [White and Falkland, 2010, Ayers and Vacher, 1986]. The interface between these two deposits typically occurs between 15 – 25 meters below sea level [Ayers and Vacher, 1986]. At depths of several hundred metres below sea level is a volcanic foundation which is remnant of an atoll's volcanic origins [Falkland and Woodroffe, 1997, Vacher and Quinn, 2004].

Atolls typically contain a reef rim with an enclosed lagoon [Mallik, 1999]. An atoll rim is formed by coral marine invertebrates of the phylum Cnidaria of which there are thousands of species [Kayanne, 2016]. Coral invertebrates live in shallow, warm marine environments and secrete calcium carbonate ( $\text{CaCO}_3$ ) to form compact colonies termed reefs [Mallik, 1999]. Coral sediments are commonly not only composed of coral reef fragments but can also include non-reef building organisms for example foraminifera, molluscs (gastropods), sea urchins, algae, and sponges.

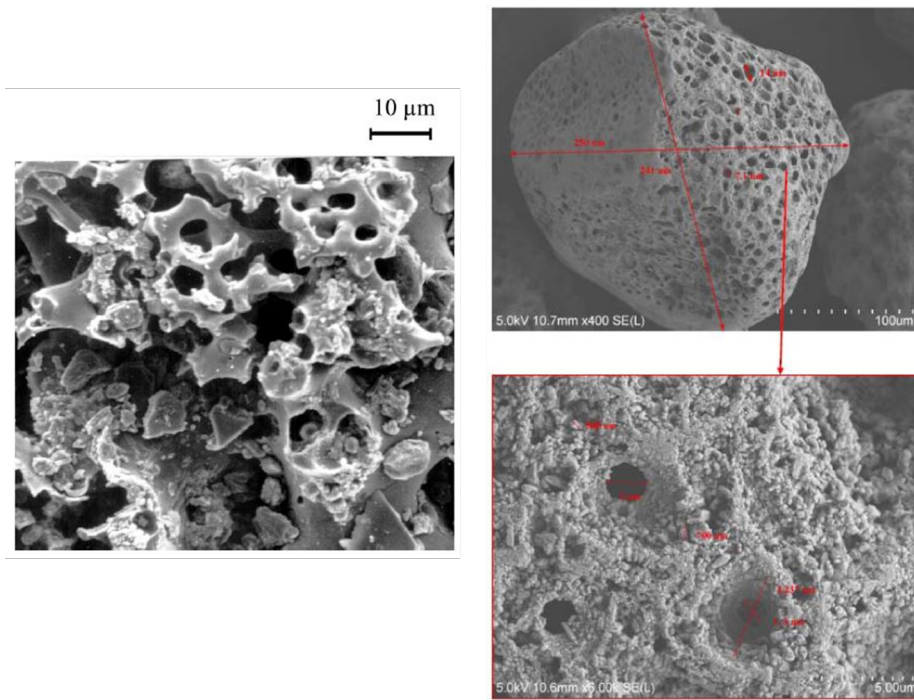
### 2.3.3. Physio-chemical properties

The dominant mineral composition of coral sediments is calcium carbonate ( $\text{CaCO}_3$ ) as shown in Figure 2.2 [Stanley, 2001]. Organisms secrete  $\text{CaCO}_3$  as either calcite or aragonite with the mineralogical difference being their crystal structures [Morrison, 1990]. Calcite forms rhombohedral crystals while aragonite forms orthorhombic crystals [Stanley, 2001]. These two minerals are distinguished by optical X-ray analysis. According to Stanley [2001] aragonite precipitates more readily in warm seawaters which are supersaturated with  $\text{CaCO}_3$  but is less stable in cooler waters and in freshwater. Aragonite is also structurally stronger than calcite [Stanley, 2001].



**Figure 2.2.:** Calcium carbonate ( $\text{CaCO}_3$ ) ionic bond.

Scanning Electron Microscopy (SEM) images of coral sand grains from Rasheed et al. [2003] and Vithanage et al. [2012] reveal a highly complex and irregular surface with multiple secondary pore spaces as a result of small fissures, channels and crevices (Figure 2.3). The marine nutrient cycling studies of Rasheed et al. [2003] and Wild et al. [2006] demonstrate that calcareous sands are more reactive than silica sands of the same grain size due to the higher specific surface area of calcareous sands. Calcareous sands from the Northern Red Sea [Rasheed et al., 2003] and Heron Island [Wild et al., 2004] measured porosities of 45% and 44% respectively, and total organic carbon contents were 0.36% and 0.18 – 0.24%, respectively. The high permeability and highly porous nature of coral sand along with its large specific surface area also makes it an efficient sorbent [Wild et al., 2006]. These characteristics of coral sand make it potentially useful in the microbial removal of microorganisms within effluent disposal fields.



**Figure 2.3.:** Image of a SEM of coral sand used in Rasheed et al. [2003] sourced from the Gulf of Aqaba, Red Sea (left) and Vithanage et al. [2012]) sourced from Lanikai Beach, Hawaii (right). All these images show a network of small fissures, channels and crevices resulting in coral sand having a large surface area.

### 2.3.4. South Tarawa coral sand

South Tarawa atoll is built from sediments that are entirely calcareous [Woodroffe and Biribo, 2011]. These sediments are derived from the skeletal fragments of organisms living on the reef such as coral, coralline or calcifying algae, molluscs and foraminifera [Woodroffe and Biribo, 2011]. Reef forming foraminifera such as *Calcarina*, *Amphistegina* and *Baculogypsina*, which are produced on the ocean side reef crest and the reef flat close to the crest are the major contributors to the coral sands of Kiribati and also Tuvalu and the Marshall Islands [Falkland and Woodroffe, 1997, Woodroffe and Biribo, 2011]. The South Tarawa seaward reef also includes *Heliopora* coral (blue coral) which is found from the seaward upper reef slope to the flat reef platform [UNEP/IUCN, 1988]. *Heliopora* coral is more abundant in the equatorial central Pacific in places such as the Gilbert Island group of Kiribati [UNEP/IUCN, 1988].

As observed by Weber and Woodhead [1972], the carbonate sands on the seaward side of South Tarawa have a much higher degree of roundness and polish than the

lagoon side carbonate sands. Falkland [1991] observed that finer sediments were deposited on the lagoon side compared to coarser sediments on the ocean side. The ocean side coral sands have been found to be dominated by magnesium calcite with aragonite being a minor component [Weber and Woodhead, 1972, Morrison, 1990]. This is supported by analysed samples taken from a transect from the edge of the lagoon to Bikenibeu beach (South Tarawa) on the ocean side which found a regular increase in the aragonite content from 50 – 84% [Weber and Woodhead, 1972].

It is hypothesised that coral sand sourced from the seaward beaches of South Tarawa may provide effective and uniform microbial removal of microorganisms found in domestic effluent. An important distinction needs to be made between coral beach sand and the coral sand substrate that forms the land mass of South Tarawa as studied in Burbery et al. [2015]. Coral beach sand is well sorted while coral sand substrate is poorly sorted and more heterogeneous with a higher proportion of silt and gravel. Studies in laboratory columns have shown that bacterial attenuation is greatest in soils with a high percentage of fine particles (i.e. clay and silt) [Tare and Bokil, 1982, O’Luanaigh et al., 2012]. This indicates that coral sand sourced from the substrate of South Tarawa may provide an insitu effective microbial removal medium but the presence of preferential pathways, caused by large bedded coral clasts, within the substrate of South Tarawa means that microbial removal cannot be uniformly guaranteed. Coral sand however, sourced from a seaward beach, would give consistent and uniform microbial removal due to its well sorted properties.

Very little research has investigated the natural properties of coral sand in general. UNEP/IUCN [1988] acknowledges that coral reef related research in the Pacific, apart from eastern Australia, lags behind that of other regions of the world due to inaccessibility and the Pacific’s vast area. Research with regards to the ability of coral sand to be utilised in solving some of the major issues in Kiribati, such as the treatment of domestic effluent, has been largely unexplored apart from a few studies which are summarised in Section 2.5.



## 2.4. Treatment of domestic effluent using granular porous media

Slow sand filtration technology for the treatment of drinking water has been employed since the 19th century but has also been shown to provide effective treatment of domestic effluent with high microbial loads as well [Pfannes et al., 2015, Metcalf et al., 2003]. Various porous media, such as silica and pumice sand, have been progressively researched for their ability to remove microbes sourced from domestic effluent [Wall et al., 2008, Pang, 2009, Weaver et al., 2013, Seeger et al., 2016, Bauer et al., 2011, Hijnen et al., 2004, DeFlaun et al., 1997]. Wastewater technologies that utilise porous media as trickling filter beds are most commonly used for the purpose of removing colloidal matter between 1 – 1000 nm during the secondary treatment phase [Metcalf et al., 2003]. Porous media is also used in the treatment of on-site domestic effluent using filter beds as a secondary treatment process. Onsite disposal of effluent is normally by subsurface soakage and assimilation into the natural soil but engineered solutions including several trenches or soakage beds filled with graded porous media are also possible [UNEP, 2002]. These types of on-site sanitation solutions require adequate area to dispose in order to not contaminate fresh water resources or marine environments [UNEP, 2002]. Filter beds are constructed to a certain depth based on the type of porous media used often with buried distribution lines which apply the effluent to the porous media at a controlled rate [Metcalf et al., 2003].

The microbial removal rates in various sediments have been summarised by Pang [2009]. Pang [2009] estimates that on average for every  $\log_{10}$  reduction in microbial concentration requires a granular porous media depth of between 0.2 – 0.6 meters but very fine sand and pumice sands only require 0.1 meter depth [Pang, 2009]. Allophanic and pumice soils contain the greatest capacity to remove bacteria and viruses because of their large surface area and positive net charge resulting in the attachment of negatively charged microorganisms [Schijven et al., 2017]. Microbial removal rates are less in soils which contain gravel sized particles which can lead to microbes being transported rapidly through macropores [Schijven et al., 2017]. Despite these estimates and the long use of slow sand filtration as water and sanitation treatment solutions, the mechanisms for pathogen removal are still poorly understood [Pfannes et al., 2015].

### 2.4.1. Microbial removal mechanisms

Microbial removal under unsaturated conditions is defined as the logarithmic reduction in microorganism concentration per transport distance in the porous media. The process of removal is achieved by any combination of physical straining, attachment/adsorption, die-off, and/or predation [Schijven et al., 2017]. Straining involves the physical filtration of microorganisms between adjacent grains that are too narrow to permit passage of the microbe [Xu et al., 2006]. Straining provides the opportunity for microorganisms to become inactivated or to die-off. Straining of microorganisms is an irreversible and travel distance-dependent process [Bradford and Kim, 2012].

Adsorption involves the attachment of microbes to the solid surface of porous media [Schijven et al., 2017]. Attachment occurs when the surface of the porous media and the microorganism have opposite electric charges [Schijven et al., 2017]. Attachment of microbes can, however, be reversed if conditions change (i.e. changes in pH) and pose a risk to receiving environments if the microorganisms are still viable and capable of infection. Typically, poorly attaching microorganisms have a low isoelectric point, which is an indication of the surface charge of a solid particle [Schijven et al., 2017].

The die-off and predation of microorganisms are determined by various abiotic and biotic environmental stresses. Abiotic stresses include temperature variations, sunlight inactivation, carbon starvation, pH fluctuation and osmotic stress from salinity changes and are well researched and understood [Feng et al., 2010]. The biotic stresses, such as protozoan predation, phage infection and bacterial antagonism and competition for nutrients from indigenous microorganisms, have been shown to be just as important. They are, however, less researched and understood due to the dynamic nature of biological interactions and technical difficulties involved in studying them [Feng et al., 2010, Pfannes et al., 2015].

Studies have investigated the combined effects of physical straining, biological and absorption mechanisms in the removal of microorganisms within porous media. According to O'Lunaigh et al. [2012], whose research investigated the attenuation capacity of highly permeable subsoils receiving both septic tank and secondary treated on-site domestic effluent, the greatest removal of *E. coli* occurred within the first 0.35 metres. This was in part attributed to the formation of a biologically active layer or biofilm-like layer called the *Schmutzdecke* (German

for 'dirt layer') that had formed on the top most layer of the unsaturated percolation trenches [Seeger et al., 2016]. It is suggested by Pfannes et al. [2015] that this layer is formed by the sorption of organic matter to granular porous media. Adin [2003], Hendricks [1991] and Wotton [2002] observed that within the *Schmutzdecke* layer(s) more than 90% of the microbial removal took place. The importance therefore of this biological layer(s) cannot be overlooked and plays an important role in assisting microbial attenuation by containing a complex mix of biofilm and biological material which provides many and diverse potential sorption sites [O'Luanaigh et al., 2012]. This layer(s) also acts to decrease the flow rate through the granular porous media and subsequently increases the retention times and opportunities for microbial attachment [O'Luanaigh et al., 2012, Elliott et al., 2015].

These results demonstrate the importance of *schmutzdecke* growth for increasing microbial reductions through (1) physical straining and/or (2) decreasing flow rate (caused by increased head loss) leading to more efficient depth filtration.

There are however negative implications for the presence of a biological layer such as a *Schmutzdecke* which is closely related to the organic content of the applied effluent. Zhuang and Jin [2003] observed that the presence of organic matter reduced virus attachment and in turn facilitated the transport of viruses by either providing additional negative charges, covering positively charged sites or competing with viruses for attachment sites. These processes are termed hydrophilic blocking or electrostatic repulsion which facilitates the transport of viruses [Zhuang and Jin, 2003].

### 2.4.2. Laboratory columns

Microbial removal rates from porous media can be determined from either field studies or laboratory columns. Laboratory column intact sediment cores or disturbed porous media cores, under saturated and unsaturated conditions, have been used widely to evaluate the fate and transport of a variety of contaminants in solution including pathogenic microorganisms [Lewis and Sjoestrom, 2010]. Column studies on the chemistry and movement of solutes within porous media began in the early 20th century with a vast amount of studies being published since 1950 [Lewis and Sjoestrom, 2010].

Microbial removal rate data are more numerous for laboratory columns than field studies [Schijven et al., 2017]. Pang [2009] cautioned that removal rates from laboratory columns are not representative of field conditions because microbial transport is hugely affected by the physical and chemical properties associated with porous media heterogeneity and transport scale. These factors are very difficult to replicate in the laboratory [Schijven et al., 2017]. This results in microbial removal estimates from laboratory columns which are one to three orders of magnitude greater than what would have been determined from the field [Engstrom and Liu, 2015, Pang, 2009]. Despite the limitations of laboratory column studies, in comparison to field studies, they are considered a pragmatic and useful tool as an initial assessment of the removal rates and processes of pathogenic microorganisms within porous granular media [Tufenkji, 2007].

### 2.4.3. Fate and transport of microorganisms in porous media

Laboratory column experiments have been undertaken previously to predict and model microbial transport through porous media. This is driven from the desire to predict the risk of transport of pathogenic microorganisms into drinking water supplies. Bacterial indicators are used because pathogens themselves are present sporadically and are often difficult to isolate. *Escherchia coli* (*E. coli*) has been used as an indicator for faecal contaminants for approximately 100 years [NRC, 2004]. The biological attributes required for an indicator organism have been defined and are [NRC, 2004];

- correlated to health risk,
- similar (or greater) survival to pathogens,
- similar (or greater) transport to pathogens,
- present in greater numbers than pathogens,
- specific to a faecal source or identifiable as to source of origin.

Microbial tracers are applied to porous granular media to determine a natural materials ability to remove microorganisms. To examine the risk associated with the transport of pathogenic microorganisms within porous granular media, model pathogenic organisms are used in experiments and are typically chosen for their conservative behaviour, in that they have poor attachment and high persistence

[Schijven et al., 2017]. Microorganisms each contain different characteristics which may be used to explain their observed transport behaviour within porous media. Transport behaviour and persistent viability of microorganisms can be influenced by whether an organism is gram-negative (i.e. *E. coli*) or gram-positive (i.e. *Enterococci*). The difference between the highly persistent gram-negative and gram-positive microorganisms are their respective outermost membrane/wall layers. Gram-negative microorganisms have an additional membrane [Qiao et al., 2012]. Other characteristics which can influence the transport of microorganisms are their shape (i.e. rod, spherical, isohedral), or the presence or absence of flagella [Schijven et al., 2017]. Flagella are the thread appendage which enables microorganisms to “swim” (motile). The presence of flagella, one or multiple, may increase their transport distance within porous media [Bergstrom, 2000].

*E. coli* and *Enterococci faecalis* (*E. faecalis*), are commonly used as effluent and water quality indicators [Metcalf et al., 2003]. Bacterial indicators such as these are considered to be conservative tracer organisms and good indicators of pathogenic faecal contamination [Metcalf et al., 2003].

Recently, research has shown the disparity between bacterial indicators and virus transport [Payment and Locas, 2011]. Alternatives have been suggested such as bacteriophages. Bacteriophages are a safe laboratory indicator virus that cannot infect a person, rather they need a specific bacterial cell for multiplication [Schijven et al., 2017]. They are also used as a viral indicator because of their structural resemblance to many human enteric viruses [Yates and Yates, 1989]. Examples of viral microbial indicators include MS2 bacteriophage (MS2 phage) and PRD1 bacteriophage. Both of these indicator viruses are considered to be conservative due to their poor ability to adsorb to porous media and their stability over a range of temperatures [Harvey and Ryan, 2004, Schijven et al., 2017, Pang, 2014]. Bacteriophages are however only a surrogate and cannot wholly represent the wide range of pathogenic viruses present in sewage which are greater than 100 species [Mara and Horan, 2003].

Even though MS2 bacteriophage does provide a worst case scenario for virus removal within porous media the use of pathogenic viruses in experiments reduces the uncertainty within a risk based assessment and ultimately better facilitates

the design of any engineered wastewater treatment solutions [Pang, 2014]. The inclusion of pathogenic viruses, such as enteroviruses, adenoviruses, astroviruses and caliciviruses, and indicator viruses within an experiment can provide an opportunity to investigate if both groups of viruses behave in a similar way. The main reason however for the inclusion of viral pathogens is to see how each viral pathogen of concern behaves with the porous media. This is important due to the wide ranging differences in characteristics and behaviours of various pathogenic viruses because of such factors as size, shape, surface charge [Schijven et al., 2017]. The inclusion of pathogenic viruses in experiments does however pose a health risk and is expensive which has limited the current level of progression and understanding of their removal within porous media [Pang, 2014]

## 2.5. Treatment of domestic effluent using coral sand

### 2.5.1. Relevant microbial removal studies

Little research has been conducted on the ability of coral beach sands to attenuate microorganisms sourced from domestic effluent. The relationship between the physio-chemical properties of coral sand and its effect on microbial transport is therefore poorly understood. The few studies that have investigated coral beach sands as a natural porous filter for microorganisms found in domestic effluent have only looked at a few bacterial and viral indicators. The studies of Vithanage et al. [2012], Firouzi et al. [2015] and Burbery et al. [2015] applied microorganisms to disturbed and undisturbed calcareous porous media in laboratory columns to determine their fate and transport and are of key relevance to this research.

Vithanage et al. [2012] investigated the transport of the microbial pathogen *Vibrio parahaemolyticus* under saturated flow conditions in coral sand from Lanikai Beach, Hawaii. The porous media properties of the three types of sand were then related to the persistence of *Vibrio parahaemolyticus*. *Vibrio parahaemolyticus* is a gram negative, rod shaped bacteria with one flagellum and is motile. It is predominantly found in saline waters and when ingested causes gastrointestinal illness. For each sand type four replicate stainless steel columns were used with column dimensions of an inner diameter 47.5 mm and 76 mm long resulting in a diameter to length ratio of 1.6. For all sand types less than 0.4% of the bacteria applied to the columns

was eluted. Vithanage et al. [2012] reported that observed bacterial transport measurements were not supported by Zeta Potential (ZP) measurements which give an indication of the surface charge at a certain pH. Scanning Electron Microscope (SEM) images clearly show that coral sands have a highly complex surface structure [Vithanage et al., 2012].

Firouzi et al. [2015] quantitatively assessed the transport of *Pseudomonas fluorescens* bacteria and deposition under saturated flow conditions in calcareous soil. *Pseudomonas fluorescens* is a common gram-negative, rod-shaped bacteria with multiple flagella and is motile. The two laboratory PVC columns were undisturbed calcareous soils from Lorestan Province, Iran. Column dimensions were an inner diameter of 80 mm and 100 mm long resulting in a diameter to length ratio of 1.25. The microbial tracers were applied to the columns under saturated conditions from the top of the columns. A high reduction rate of bacteria was observed and attributed to the calcium carbonate within the soil which has favourable attachment sites for bacteria [Firouzi et al., 2015]. Firouzi et al. [2015] concluded that at a neutral pH (7.4 – 7.52), calcite appears to have effective positive attachment sites for negatively charged colloids. Results from this study show that soil mineralogy, and to a lesser extent water velocity, have a significant influence on the attachment rate and removal of bacteria [Firouzi et al., 2015].

The other published study on the microbial transport in coral sand media through short laboratory columns is that of Burbery et al. [2015]. Burbery et al. [2015] investigated the effective removal rates of *E. coli* J6-2 bacteria and F-RNA MS2 bacteriophage (MS2 phage). The three laboratory PVC columns were packed with disturbed coral sand material sampled from the top of the Bonriki Freshwater Reserve, South Tarawa, Kiribati. Column dimensions were an inner diameter of 27 mm and 146 mm long resulting in a diameter to length ratio of 5.4. The microbial tracers were applied to the columns under saturated flow conditions from the base of the columns. Similar to Vithanage et al. [2012] the results from Burbery et al. [2015] tend to indicate that as a natural porous medium, coral sand is relatively effective at filtering bacteria. The preliminary findings of Burbery et al. [2015] indicated that coral sand is much less effective at virus removal. The coral sand from the Bonriki Freshwater Reserve was very heterogeneous and would be expected to provide less filtration compared to a homogeneous well sorted beach sand as was used in Vithanage et al. [2012].

### 2.5.2. Onsite domestic effluent drainage fields

There have been on-going efforts to improve sanitation practices on the atolls of Kiribati with alternative modes of sewage disposal being explored. The limited resources of many atolls, however, restrict the available sanitation options. As previously mentioned in Section 2.2.4 engineered effluent treatment solutions should be affordable, sustainable and utilise technology which is locally available and easily maintained.

In places such as South Tarawa, Kiribati, septic tank on-site wastewater treatment systems are being proposed as a simple, cost-effective way to improve wastewater management at a household level. It is the effluent drainage field of a septic tank system that largely determines its effectiveness at mitigating microbial loads to the underlying freshwater lenses.

Of relevance to this research is a study conducted by Tait et al. [2013] which provided an example of a simple engineered solution for the secondary treatment of effluent from household septic tanks in the Cook Islands. In Tait et al. [2013] novel design, effluent from a septic tank is drained through a coconut husk filter to a saturated trench, before discharging to the carbonate sand substrate. The research showed the system enhanced nutrient Nitrogen (N) and Phosphorus (P) removal from wastewater, but did not explicitly examine whether it offers any heightened pathogen removal [Tait et al., 2013].

No relevant studies currently exist on the microbial removal properties of seaward Kiribati coral beach sand. There is a need therefore for scientifically based evidence to determine the microbial removal properties of coral beach sands sourced from Kiribati and how these sands might be incorporated into the design of onsite domestic effluent drainage beds. This research aims to begin the process of filling this knowledge gap.



### 3. Physio-chemical properties of coral sand

This chapter describes the methods and materials employed to determine the physio-chemical properties of coral sand used in this research by various physical and chemical tests. The results of these tests are also presented and discussed.

#### 3.1. Particle Size Distribution

Coral sand was sourced from Bikenibeu beach, on the ocean-facing, southern side of South Tarawa, Kiribati ( $1.367^{\circ}\text{N}$ ,  $173.126^{\circ}\text{E}$ ) as shown in Figure 3.1. The coral sand was collected from the foreshore as shown in Figure 3.2.

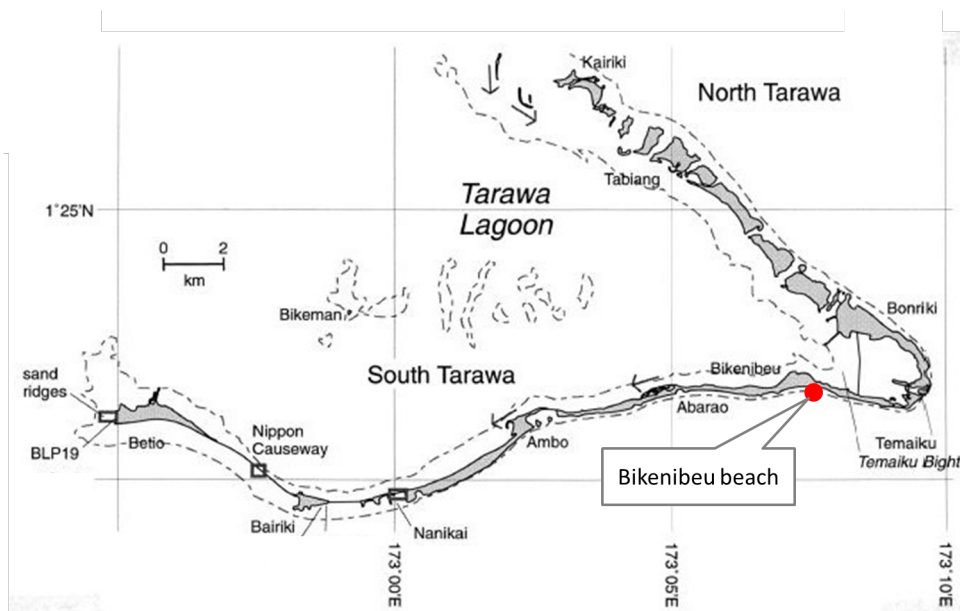


Figure 3.1.: Location of Bikenibeu beach, South Tarawa, Kiribati.



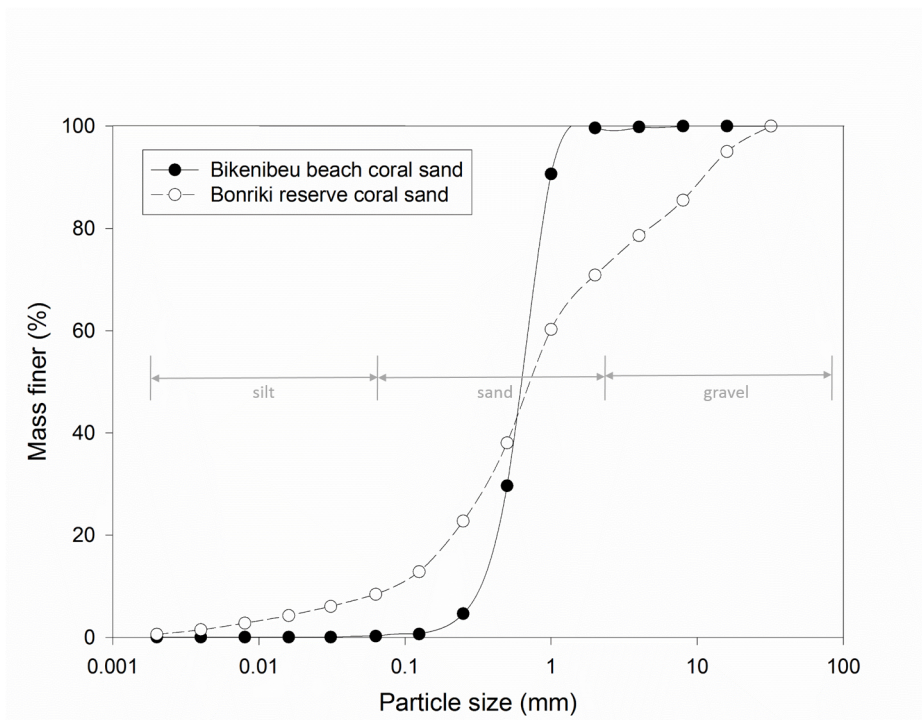
**Figure 3.2.:** Location of coral sand which was collected for this research on the foreshore of Bikenibeu beach, South Tarawa, Kiribati (authors own image).

A Particle Size Distribution (PSD) was made of the material, using dry-sieving methods (Endecotts sieves) to determine the proportions of the follow size divisions:  $>5$  mm; 2 mm; 1 mm; 0.5 mm; 0.250 mm; 0.125 mm; 0.063 mm and  $<0.063$  mm [Wentworth, 1922]. Particle size analysis software GRADISTAT was used to calculate the particle size statistics for the coral sand [Blott and Pye, 2001].

The PSD of the Bikenibeu beach coral sand used in this research revealed that just over 60% was sized between 0.5 mm and 5 mm (Table 3.1). The second largest proportion (25%) was between 0.25 mm and 0.5 mm. According to GRADISTAT the sand was classified as a moderately well sorted coarse sand with the PSD curve shown in Figure 3.3. For comparison, Figure 3.3 provides the PSD curve for the Bonriki freshwater reserve coral sand used in Burberry et al. [2015] which was a poorly sorted sandy medium gravel.

Particle size (mm)	Aggregate class	Wentworth scale (% w/w)	Average Zeta Potential (mV) $\pm$ Standard Deviation
>5	fine gravel	0.15	$-7.3 \pm 0.49$
>2	very fine gravel	0.2	$-6.1 \pm 1.36$
>1	very coarse sand	9.3	$-5.0 \pm 2.19$
>0.5	coarse sand	61.2	$-7.2 \pm 5.08$
>0.25	medium sand	25.3	$-7.7 \pm 2.98$
>0.125	fine sand	3.7	$-10.9 \pm 6.02$
>0.063	very fine sand	0.04	$-5.4 \pm 6.22$
<0.063	silt	0.02	$-9.2 \pm 7.33$

**Table 3.1.:** Particle Size Distribution (PSD) of Bikenibeu beach coral sand sourced from South Tarawa, Kiribati. Class sizes and descriptions relate to the Wentworth scale (w/w). Each particle size fraction was analysed in triplicate to achieve an average Zeta Potential (mV) with the standard deviation.



**Figure 3.3.:** Particle Size Distribution (PSD) for the Bikenibeu beach coral sand used in this research. The PSD curve for coral sand sampled from 200 - 400 mm depth on the Bonriki reserve and examined in Burbery et al. [2015] is shown for comparison.

Langenbach et al. [2009, 2010] investigated the removal of *E. coli* and enterococci using various sand grain sizes and found that fine grained and uniform sand with a high sand surface area achieved the best removal under low flow conditions. Supported by Langenbach et al. [2009, 2010] the results presented by this research suggest that the coral sand from Bikenibeu beach would be better suited for microbial removal within an engineered septic tank effluent disposal field rather than the more poorly sorted Bonriki coral sand sediments. The free draining and uniform grain size of the Bikenibeu beach coral sand that was used in a engineered septic tank disposal field would potentially avoid the issues of preferential flow while also reducing the uncertainties surrounding the removal of pathogens which would aid the protection of the underlying groundwater lenses.

## 3.2. Zeta Potential

As an approximate measure of the surface potential of the coral sand, the Zeta Potential (ZP) of discrete sediment size fractions were measured (classified by the above sieving method outlined in Section 3.1), and at the pH level representative of septic effluent used in the experiments. For this, 2 g of sediment from each particle size fraction was sonicated in 18 mL of pH 8 saline solution for 2 minutes before ZP analysis was made on the (0.22  $\mu\text{m}$ ) filtered solution using laser Doppler microelectrophoresis (Malvern Instruments Zetasizer Nano ZS). Methods for the ZP analysis were based upon several key studies but no standard procedure currently exists [Pang, 2009, Kaya and Yukselen, 2005, Yukselen and Kaya, 2003, Stephan and Chase, 2001]

Analysis of the Bikenibeu beach coral sand's ZP found no correlation between ZP and the sediment grain size fractions, as classified by the PSD analysis (Table 3.1). For the test conditions of coral sand, which was suspended in saline solution at pH 8, the average ZP of the coral sand was measured to be -7.4 mV within a range of -17.5 to -0.2 mV (Table 3.1).

## 3.3. Geochemistry

The mineral composition of the Bikenibeu beach coral sand was determined by X-ray Powder Diffraction (XRD), using a Phillips PW1820/1710 diffractometer at the

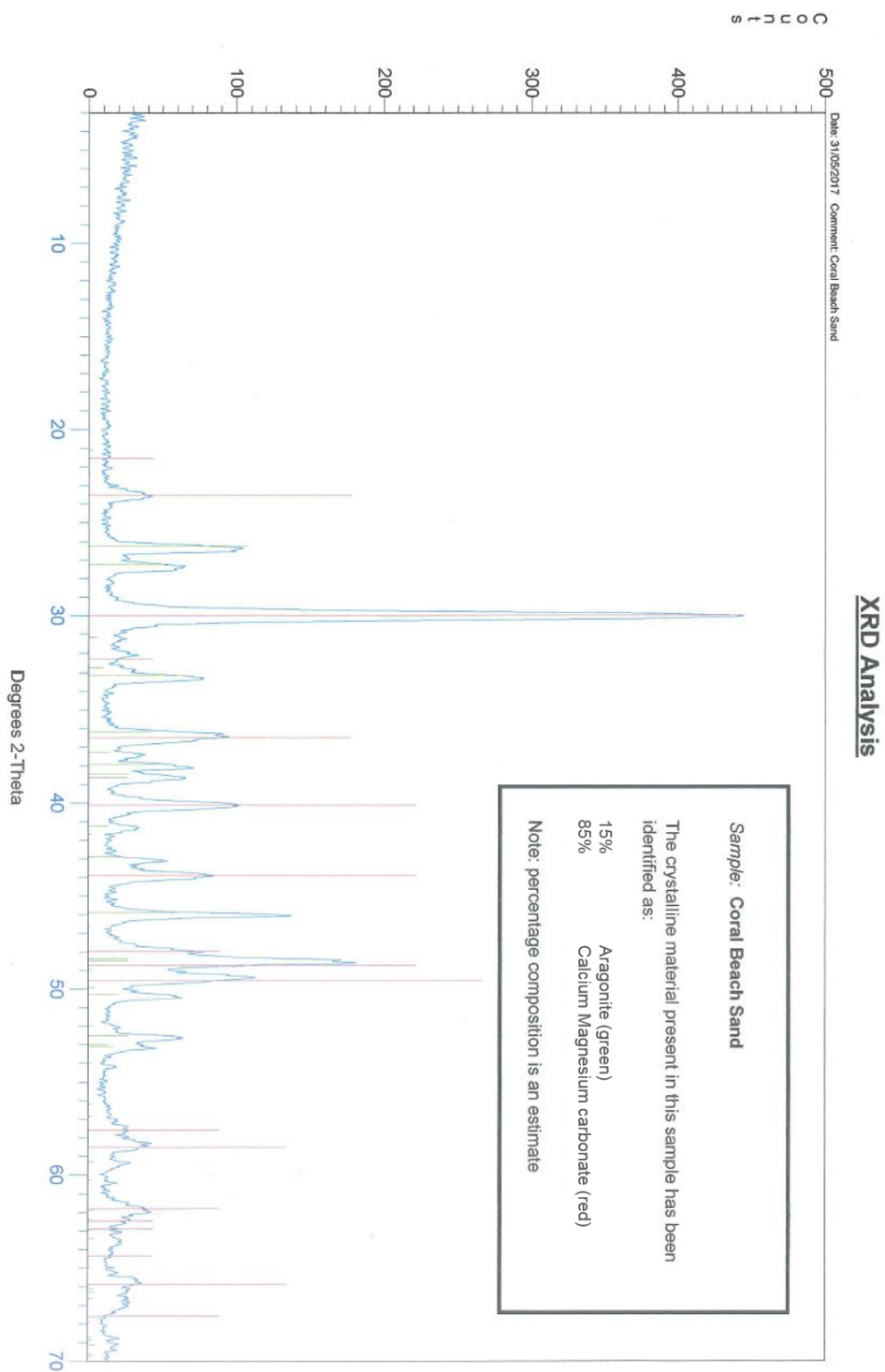
University of Canterbury Geology Department. A generic sample of coral sand was ground using mortar and pestle until a marked decrease in friction was experienced. The powder was then ‘loosened’ to remove any clustering and fixed to a silicon single crystal substrate using a minimal amount of amorphous vaseline. The mineral identification of the coral sand was achieved by analysing its crystalline structure at various angles and comparing the wavelength results to known values.

The XRD analysis revealed the mineral composition of the coral sand to be 85% calcium magnesium carbonate ( $\text{CaMg}(\text{CO}_3)_2$ ) and 15% aragonite ( $\text{CaCO}_3$ ). The XRD diffractogram is included in Figure 3.4. These results support the findings of Weber and Woodhead [1972] and Morrison [1990] in that the ocean side coral sands have been found to be dominated by magnesium calcite with aragonite being a minor component. This highlights that there are spatial differences in the geochemistry of lagoon and ocean side coral sand sediments.

## 3.4. Scanning Electron Microscope

The surface of the coral sand was imaged using a Phenon ProX Scanning Electron Microscope (SEM). A generic sample of Bikenibeu beach coral sand was examined and is shown in Figure 3.5. The image indicates that coral sand has a highly rough and large surface area as visible by multiple micropores.

For comparison, Figure 3.6 shows an image of pumice sand grain from Wall et al. [2008]. The SEM image of Wall et al. [2008] shows that pumice has both intra (i.e. blind pores) and inter-particle voids. Wall et al. [2008] found that at a field trial site in Rotorua (New Zealand) a high removal (>99%) of the microbial indicators MS2 bacteriophage and *E. coli* were observed within pumice sand. Wall et al. [2008] concluded that pumice sand is an effective sorbent with the ability to retain microbial contaminants due to its high surface area and surface properties. Since pumice sand and coral sand (based on a visual analysis) appear to share similar surface property characteristics, the results of Wall et al. [2008] may indicate that the physical structure of coral sand could be beneficial for microbial removal.

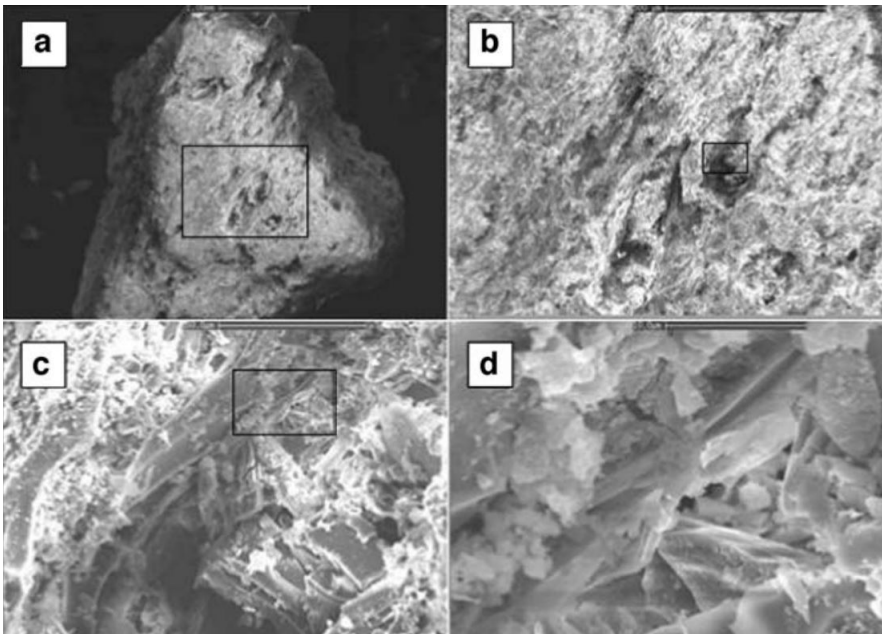


**Figure 3.4.:** Diffractogram from the XRD analysis of Bikenibeu beach coral sand.





**Figure 3.5.:** Scanning Electron Microscope (SEM) image of Bikenibeu beach coral sand (authors own image).



**Figure 3.6.:** Porosity of a 2 mm pumice sand grain from Rotorua (New Zealand) at four different scales (**a - d**) by SEM. The inset box indicates the location of the subsequent image [Wall et al., 2008].

### 3.5. Unsaturated hydraulic conductivity

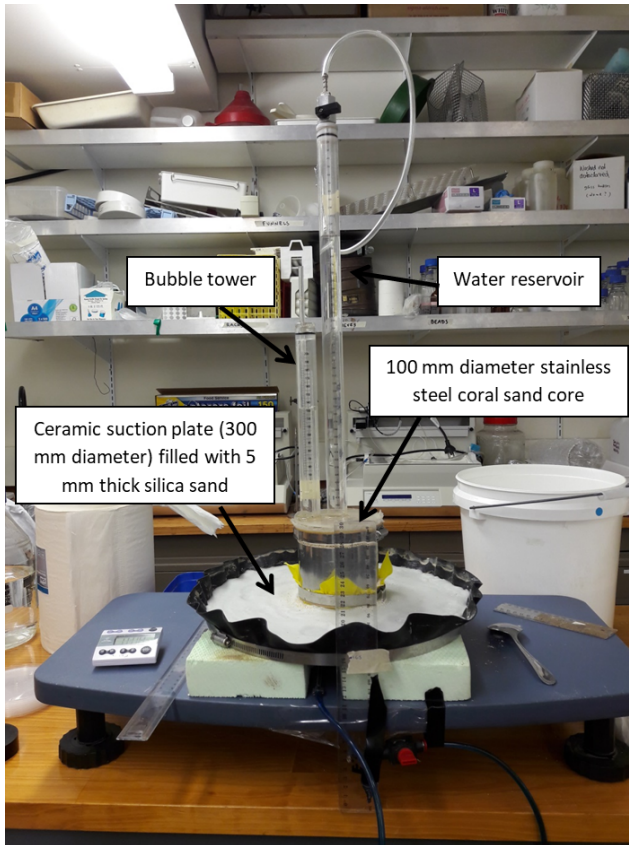
An estimate of the unsaturated hydraulic conductivity of coral sand was obtained using a tension infiltrometer. Method 10.4 for small (50 - 100 mm diameter) cores found in McKenzie et al. [2002], was used for a 100 mm diameter by 74 mm long stainless steel core of coral sand (Figure 3.7). The core of coral sand contained the same particle size distribution as was used in all the column experiments presented in this research (Section 3.1). To achieve uniform packing, the core was filled following procedures recommended by Oliveira et al. [1996]. That is, they were progressively filled with sand added in 20 g aliquots or incremental lifts of less than 2 mm. After each amendment, the core apparatus was gently vibrated and the sand tamped, using a comparable force each time. A porous mesh (63  $\mu\text{m}$ ) was secured to the base of the stainless steel core by a thick rubber band to hold the sand within the core.

A 300 mm diameter ceramic suction plate was covered with a thin (approximately 5 mm) layer of silica sand. The ceramic suction plate covered with silica sand was set up to create a surface suction equal to that which was applied by the tension infiltrometer. This was achieved by placing the tension infiltrometer directly onto the silica sand covered ceramic suction plate. The outlet tube at the base of the suction plate and the water level in the bubble tower were altered until a bubble was held at the end of the air inlet tube ensuring that top and bottom suctions were equal.

The coral sand core was placed onto the silica sand covered ceramic suction plate and gently twisted to ensure complete contact between the two. The water reservoir tower was then filled with deaerated water to a known level and placed onto the top of the coral sand core. The tension infiltrometer was gently twisted also to ensure complete contact between the infiltrometer and the core. As soon as the infiltrometer was connected to the core the experiment started. While the water reservoir tower drained, the water level within the tower was recorded along with the time since the start of the experiment. Various tensions, approaching saturation, were applied to the coral sand core via the tension infiltrometer and included 100 mm; 80 mm; 60 mm; 50 mm and 40 mm tensions. Three replicates for each tension were completed.

When unit hydraulic gradient had been obtained via the above methods, the unsaturated hydraulic conductivity was calculated using Equation 3.1.





**Figure 3.7.:** Tension infiltrometer apparatus used to estimate the unsaturated hydraulic conductivity of coral sand (authos own image).

$$K = Q/A \quad (3.1)$$

Where:

$K$  = Hydraulic conductivity (m/day)

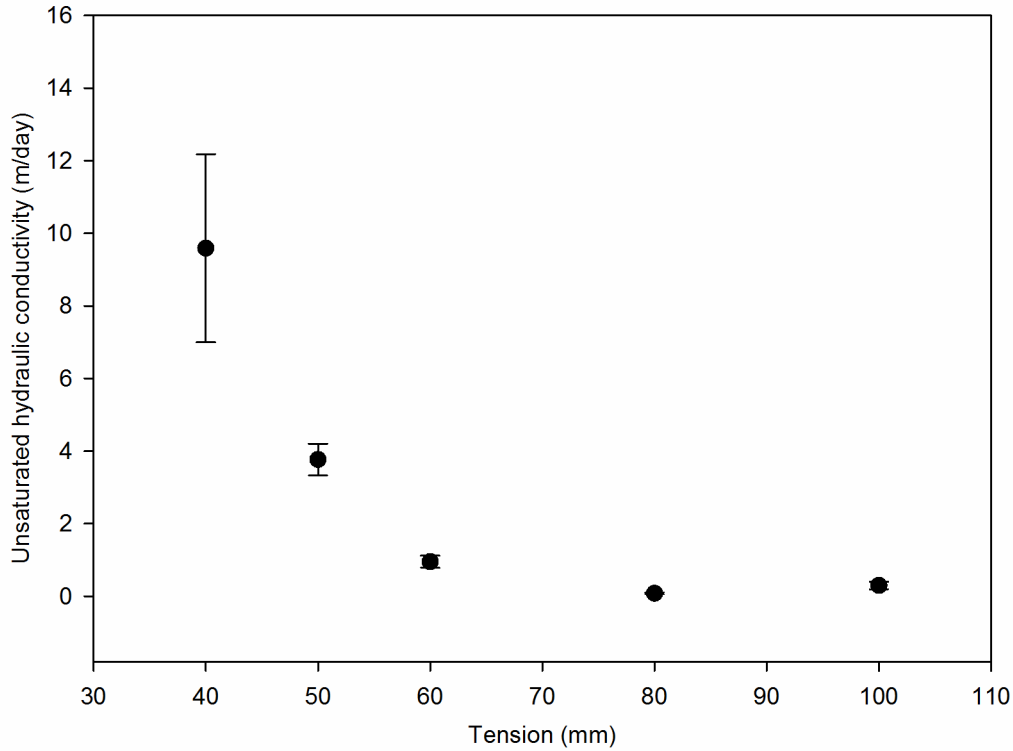
$Q$  = Flow ( $\text{m}^3/\text{day}$ )

$A$  = Area of the soil column ( $\text{m}^2$ )

The same Bikenibeu beach coral sand was packed into the stainless steel tension infiltrometer for each infiltrometer experiment at the various tensions. Multiple tests were performed under these various tensions with an average dry bulk density for every test of  $1.53 \text{ g/cm}^3$  and a total porosity of 42%. Figure 3.8 presents the

unsaturated hydraulic conductivity results under the various tensions and water contents that were applied to the infiltrometer filled with coral sand.

Conclusions from the unsaturated hydraulic conductivity could not be accurately made because a soil water characteristic curve was not determined for unsaturated coral sand.



**Figure 3.8.:** Bikenibeu beach coral sand unsaturated hydraulic conductivity results (with error bars) under various infiltrometer tensions.

### 3.6. Saturated hydraulic conductivity

Saturated hydraulic properties of the coral sand were obtained using the constant head method [Fetter, 2001]. A 275 mm long by 43.7 mm diameter PVC core was filled with coral sand of the same particle size distribution used throughout this research (Section 3.1). A porous fibre glass mesh was placed at the base of the core to hold the coral sand within the core. The coral sand was packed using the same methods as described in Section 3.5. The core was filled with deaerated water 48

hours before the start of the constant head test to ensure that the coral sand was fully saturated. A constant head of water, supplied from a header tank, was applied to the top of the core (Figure 3.9). Manometers which were near the top and bottom of the core, and 200 mm apart, were monitored over a period of 80 minutes after which steady flow through the core was achieved. Darcy's law (Equation 3.2) was rearranged and used to calculate the hydraulic conductivity under constant head by measuring the cumulative volume of water drained from the core over a period of time.

$$K = VL/Aht \quad (3.2)$$

Where:

$K$  = Hydraulic conductivity (m/day)

$V$  = Collected volume of water (m<sup>3</sup>)

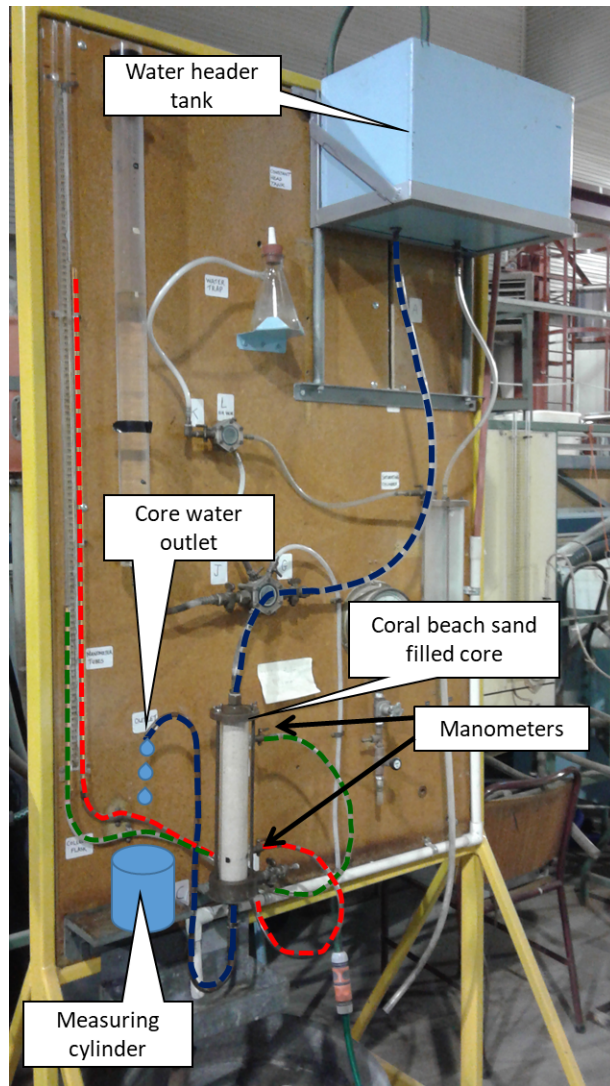
$L$  = Length of core (m)

$A$  = Cross sectional area of the soil column (m)

$\Delta h$  = Head difference between the manometers (m)

$t$  = Time required to get volume  $V$  (days)

The Bikenibeu beach coral sand that was packed into the constant head PVC core apparatus resulted in a dry bulk density of 1.48 g/cm<sup>3</sup> and a total porosity of 44%. Under the same packing conditions the test was repeated six times and resulted in an average saturated hydraulic conductivity value of 269 m/day with values ranging between 262 and 276 m/day. These hydraulic conductivity estimates would presumably enable the transport of any microbial contaminants quickly within a saturated effluent drainage field system. These results highlight the need for a controlled and low flow application of household effluent to a coral sand disposal field in order to achieve some level of treatment. The results also highlight the need to maintain unsaturated conditions within a septic tank effluent disposal field to optimise the removal of microorganisms. Unsaturated conditions would also reduce the opportunity for microbial contaminants to reach the underlying groundwater lens.



**Figure 3.9.:** Saturated hydraulic conductivity experimental apparatus using the constant head method (authors own image).

## **4. Microbial removal in clean coral sand**

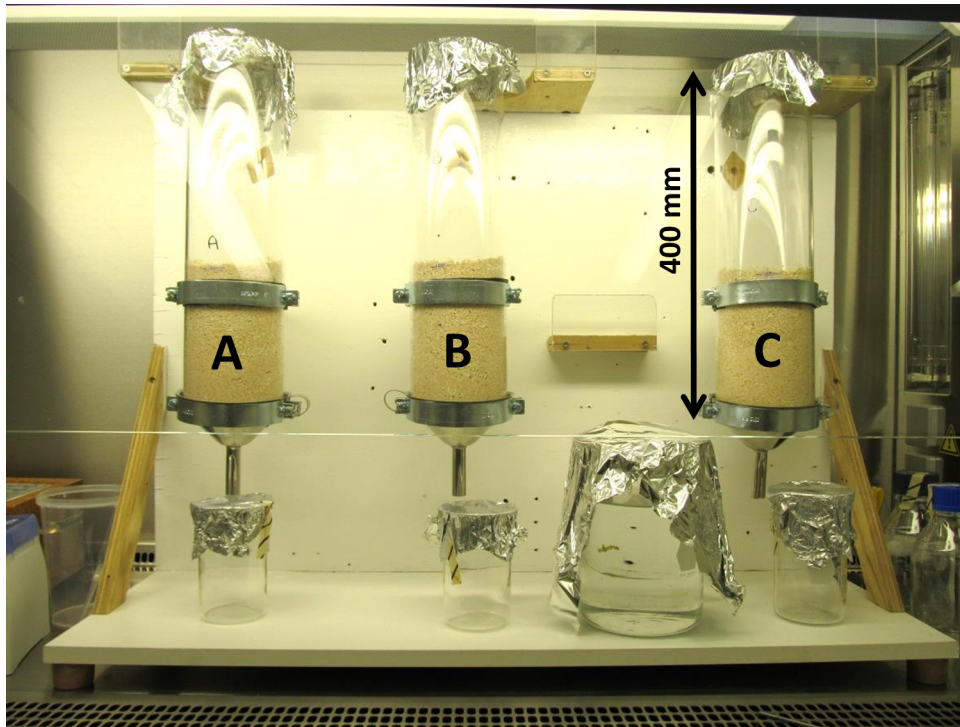
The next step after determining the physio-chemical properties of coral sand used in this research was to determine its ability to remove microorganisms in its unchanged and natural state as found on Bikenibeu beach, South Tarawa, Kiribati. This chapter describes the materials and methods employed in the unsaturated clean coral sand packed laboratory column experiments with the results presented and discussed.

### **4.1. Laboratory column apparatus**

Coral sand with a PSD as detailed in Section 3.1 was used for the clean coral sand experiments. This was done to examine coral sand which is found naturally on the ocean side beach at Bikenibeu and to assess its ability to remove microorganisms without being graded or sieved. Examining various particle sizes of coral sand grains was not explored in this research. According to Lewis and Sjoestrom [2010] utilising a graded sand for a septic tank effluent disposal field bed would potentially reduce the risks around macropores and preferential flow paths [Lewis and Sjoestrom, 2010].

To avoid altering the geochemical composition of the sand that could potentially bias the outcome of any microbial transport experiments, it was not sterilized using autoclave equipment. It was, however, over-dried at 65°C for 48 hours. As shown in Figure 4.1 the coral sand was then packed into three vertical experimental columns, to serve as model domestic septic tank effluent drain beds (referred to as columns A, B and C).

Three borosilicate-glass open ended columns 400 mm long and 106 mm in diameter were used for the experiments. A 125  $\mu\text{m}$  stainless steel mesh was glued to the base



**Figure 4.1.:** Vertical unsaturated laboratory columns A, B and C, in the process of being filled to 400 mm. These columns served as model domestic septic tank coral sand effluent drainage beds.

of each of the columns to support the sand contents and enable free drainage. To achieve uniform packing, the columns were filled following procedures recommended by Oliveira et al. [1996] as described in Section 3.5. The total thickness of coral sand packed in each column was 400 mm. According to Lewis and Sjoestrom [2010] the recommended column diameter to length ratio should be 1:4 to minimise sidewall flow effects. The columns used in this research fall within this recommended ratio.

The achieved dry bulk density and total porosity for each column are given in Table 4.1. Stainless steel funnels were positioned at the bottom of each column to capture the drainage, which was collected in dedicated sterile glass beakers. To mimic a subsurface environment, all apparatus were wrapped in foil, and for health and safety reasons the columns were housed and examined within a Class II biological safety cabinet. All operations were carried out at room temperature ( $20\pm 2^{\circ}\text{C}$ ).

Borosilicate glass and stainless steel fittings were used for the column apparatus in this research to avoid the issues that Burberry et al. [2015] encountered. Burberry et al. [2015] observed that removal rates for MS2 phage were compromised by the

use of plastic apparatus which MS2 phage formed an irreversible adsorption onto the plastic column apparatus and also possibly onto the plastic tubing used. Farkas et al. [2014] also noted that since plastic is hydrophobic (i.e. repels water) it has a high affinity for MS2 phage.

According to Lewis and Sjoestrom [2010], to assess if a coarse sand soil column has been sufficiently compacted the total porosity and dry bulk density should be between 31 - 46% and 1.8 - 1.4 g/cm<sup>3</sup>, respectively. This guideline is given for natural material which seeks to model a natural environmental setting. The coral sand packed into the columns for the clean coral sand experiment fell within the acceptable bulk density range for a sufficiently compacted column but had a lower porosity than what is recommended by Lewis and Sjoestrom [2010]. The columns were however tightly packed to examine an engineered septic tank disposal field and not to examine the microbial removal capacity of naturally deposited coral sand sediments. The columns were also tightly packed to maximise the potential for physical straining and adsorption of microorganisms by the coral sand.

	Column A	Column B	Column C
Dry bulk density (g/cm <sup>3</sup> )	1.45	1.49	1.49
Total porosity (%)	26	28	27

**Table 4.1.:** The dry bulk density and total porosity results for the Bikenibeu beach coral sand packed columns during the clean coral sand experiments.

## 4.2. Microbial properties, propagation and preparation

The following section explains the microorganisms used in the tracer experiments, their propagation and preparation before addition to the injection solution which was applied to each column. The microorganisms were:

- *Escherichia coli* J6-2 (*E. coli* J6-2)
- *Enterococci faecalis* (*E. faecalis*)
- MS2 bacteriophage (MS2 phage)
- Adenovirus

- Echovirus
- Norovirus
- Rotavirus

#### 4.2.1. Bacterial and viral indicator organisms

The bacterial microbial indicators *Escherichia coli* J6-2 (*E. coli* J6-2) and *Enterococci faecalis* (*E. faecalis*) are often used as model pathogens within experiments to determine the microbial removal ability of porous media as they are indicator organisms used commonly in effluent and water quality standards [Wang et al., 2013].

The bacterial indicator organism *E. coli* J6-2 is a non-pathogenic, lactose negative, nalidixic acid resistant derivative of *E. coli* K-12 [Sinton, 1980]. It has rod-shaped cells that are 1.0 - 1.5  $\mu\text{m}$  in diameter and 3.0 - 5.0  $\mu\text{m}$  long making it the largest microorganism used in this research (Figure 4.2) [Sinton, 1980]. *E. coli* J6-2 are gram-negative rod shaped cells that have multiple flagella and are motile [Bergstrom, 2000]. *E. coli* J6-2 was propagated by streaking cells onto MacConkey agar (Merck, Germany). Plates were grown at 37°C for 18 hours with single colonies inoculated into Tryptic Soy Broth (TSB) (Merck, Germany) and shaken at 100 rpm, 37°C for 18 hours. Cells were washed in saline (0.85%) twice by centrifuging at 3000 rpm for 15 minutes. After each spin the supernatant was aspirated off, the pellets re-suspended in saline solution and stored at 4°C for no longer than 24 hours before being added to the experiment injection solution [Weaver et al., 2013].

The bacterial microorganism *E. faecalis* was an additional indicator alongside *E. coli* due to its high resistance to stress and drying and its longer survival times [Wang et al., 2013]. *E. faecalis* is a member of the genus enterococci and like *E. coli* are commonly found in the human and animal gut. *E. faecalis* are gram-positive spherical cells that have flagella but are non-motile [Mara and Horan, 2003]. *E. faecalis* cells are coccoid or ovoid cells, 0.6 - 2.0  $\mu\text{m}$  in diameter and 0.6 - 2.5  $\mu\text{m}$  long which form chains (Figure 4.2) [Cail and Hochella, 2005]. *E. faecalis* was propagated by streaking cells onto Columbia Blood Agar (Oxoid, England). The subsequent *E. faecalis* propagation methods were the same as mentioned above for *E. coli* J6-2



and stored at 4°C for no longer than 24 hours before being added to the experiment injection solution [Weaver et al., 2013].



**Figure 4.2.:** The bacterial microbial indicators *E. coli* (left) rod-shaped cells are 1.0 - 1.5  $\mu\text{m}$ , *E. Faecalis* (middle) coccoid or ovoid cells are 0.6 - 2.0  $\mu\text{m}$  in diameter and 0.6 - 2.5  $\mu\text{m}$  in length and a generic T4 bacteriophage (right) are 0.026  $\mu\text{m}$  in diameter. Images sources: *E. coli*, [www.biocote.com](http://www.biocote.com); *E. Faecalis*, [www.medicalnewstoday.com](http://www.medicalnewstoday.com); and generic bacteriophage, [www.gizmodo.com.au](http://www.gizmodo.com.au)

Bacteriophages, like MS2 bacteriophage (MS2 phage) are often used as a surrogate to evaluate the transport and fate of pathogenic viruses [Cheng et al., 2007]. They are considered useful model microorganisms due to their similar size and structure to many enteric viruses, are non-pathogenic and relatively inexpensive [Cheng et al., 2007]. MS2 phage is icosahedral RNA phage with a diameter of approximately 0.026  $\mu\text{m}$  [Pang, 2009, Goyal and Gerba, 1979] (Figure 4.2). The host strain used for MS2 phage was *E. coli* HS (pFamp) R [Debartolomeis and Cabelli, 1991] which is resistant to ampicillin and streptomycin sulphate. MS2 phage plaques were propagated by growing plaques to confluence on overlay pour plates. The overlay was then removed, shaken vigorously in 100 mL sterile water, and then spun at 1000 xg for 25 minutes to remove cellular and agar debris. The supernatant was filtered (0.22  $\mu\text{m}$ ) and stored at -20°C for no longer than 24 hours before being added to the experiment injection solution, as per method described in Weaver et al. [2013].

### 4.2.2. Pathogenic viral organisms

The viruses, adenovirus (70 - 90 nm), echovirus (28 - 30 nm), norovirus (35 - 39 nm) and rotavirus (80 nm), are pathogenic organisms [Pang, 2009]. These viruses need a host in order to replicate and survive and when ingested by humans are capable of causing severe illness [Mara and Horan, 2003]. Adenovirus, echovirus

and rotavirus were each prepared by growing in cell lines until confluent growth was achieved (normally 3 - 5 days). Infected cell lines were washed and treated to freeze-thaw cycles before being stored at -80°C, pending use in the experiment injection solution. Viral stock titre was calculated using the TCID<sub>50</sub> procedure [Reed and Muench, 1938].

### 4.2.3. Microbial tracer injection solution preparation

To prepare the experiment tracer injection solution, separate virus stocks of adenovirus, echovirus, rotavirus and norovirus were mixed together and centrifuged at 34,500 rpm (Beckman Coulter Ultracentrifuge, Optima L-100K) for one hour at 4°C. The resulting pellets were re-suspended in a saline solution, and were shaken overnight at 120 rpm on an orbital shaker at 4°C. The viral mixture was sonicated in a water bath (Bandelin, Sonorex) at room temperature for 2 minutes, shaken for 30 minutes, and then sonicated for a further 2 minutes. The pathogenic virus mixture along with *E. coli* J6-2, *E. faecalis* and MS2 phage were added to the household effluent, vigorously shaken for 1 minute and applied immediately to the top of the columns.

## 4.3. Microbial enumeration

### 4.3.1. Bacterial and viral indicator organisms

*E. coli* J6-2 was enumerated by pour plating 1 mL aliquots (or dilutions thereof) into Brilliance TM *E. coli*/coliform selective agar (Oxoid, United Kingdom). *E. coli* J6-2 plates were incubated at 30±1°C for 4±2 hours and then 44±0.5°C for 20±4 hours. Typical colonies were enumerated by eye and the number of colony forming units (cfu) present per mL was calculated. The detection limit was 1 cfu / mL.

*E. faecalis* was enumerated by pour plating 1 mL aliquots (or dilutions thereof) into Chromocult *Enterococci* Agar (Merck, Germany). *E. faecalis* plates were incubated at 30±1°C for 4±2 hours and then 42±0.5°C for 20±4 hours. Typical colonies were enumerated by eye and the number of colony forming units (cfu) present per mL was calculated. The detection limit was 1 cfu / mL.

MS2 phage was analysed in 1 mL aliquots (or dilutions thereof) using the American Public Health Association [APHA, 1998] overlay pour plating method and the host strain *E. coli* HS (pFamp)R [Debartolomeis and Cabelli, 1991]. Plates were incubated at  $35\pm 1^{\circ}\text{C}$  for  $20\pm 4$  hours. Plaques were enumerated by eye and counts were expressed as plaque forming units (pfu) per mL. The detection limit was 1 pfu / mL.

#### 4.3.2. Pathogenic viral organisms

To enumerate the pathogenic viruses, the molecular technique Reverse Transcription quantitative Polymerase Chain Reaction (RT-PCR) was used to amplify the genetic information contained within each of the target viral organisms [Mara and Horan, 2003]. The samples were first purified using the chloroform extraction technique to remove any bacterial contaminants that could affect the PCR analysis. Samples were mixed 1:1 with chloroform, vortexed for 2 minutes, spun at  $10,000 \times g$  for 20 minutes, and the aqueous phase stored at  $-80^{\circ}\text{C}$ . Viral RNA from echovirus; rotavirus and norovirus, and DNA from adenovirus was then extracted using a High Pure Viral Nucleic Acid Kit (Roche). Finally, purified RNA was reverse-transcribed using the Invitrogen SuperScript VILO cDNA synthesis kit (Invitrogen) then amplified using 2 step real-time PCR and Platinum Quantitative PCR SuperMix-UDG (Invitrogen); DNA was amplified using real-time PCR using LightCycler 480 Probe Master reaction mix (Roche). Pathogenic virus counts were calculated as genome copies / mL.

### 4.4. Clean coral sand experiments

#### 4.4.1. Clean coral sand tracer experiment

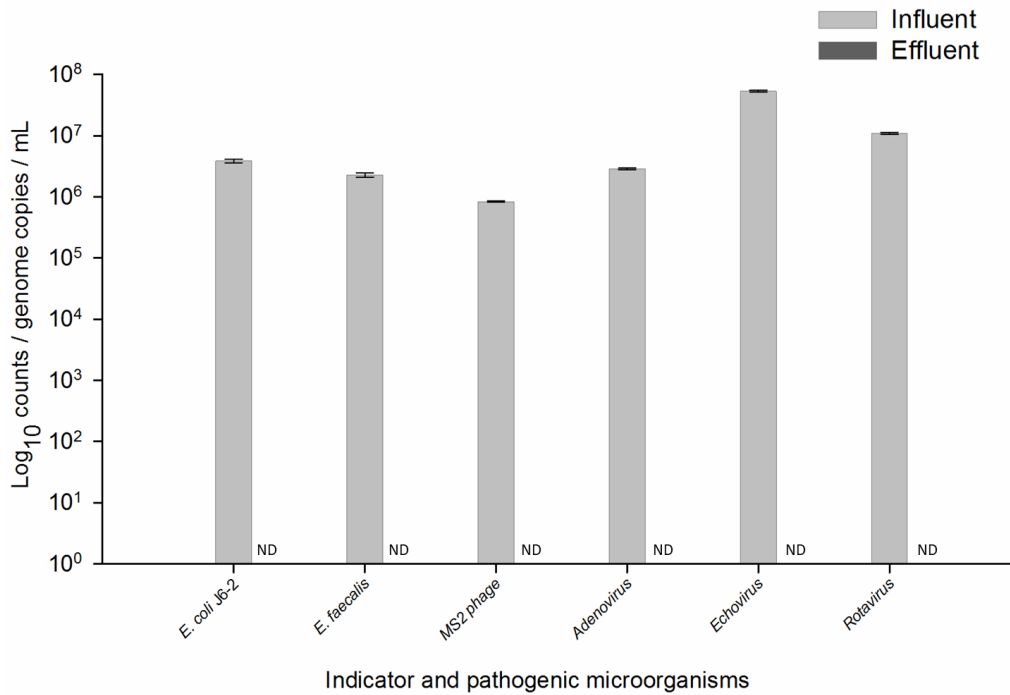
The unsaturated clean coral sand experiment simulated the ability of a newly installed coral sand effluent disposal field to attenuate pathogenic microorganisms. Since septic tank onsite wastewater disposal systems in Kiribati would most likely use groundwater to flush toilets, Artificial Ground Water (AGW) with a chemical composition similar to shallow groundwater found in Kiribati was created. The AGW was prepared from a base of sterile MilliQ water and contained 250 mg/L of

chloride (prepared from NaCl salt) at a pH of 8, which was adjusted by addition of sodium hydroxide. As an initial investigation 1 Pore Volume (PV) (1.2 L) of AGW was applied to the columns with the drainage analysed for background levels of the microbes tested in the experiments (*E. coli* J6-2; *E. faecalis*; MS2 phage, adenovirus, echovirus and rotavirus). The AGW was then used as a base for the tracer injection solution which was spiked with a suite of bacterial and viral indicator organisms (*E. coli* J6-2; *E. faecalis*; MS2 phage) and pathogenic viruses (adenovirus, echovirus, rotavirus). Norovirus was not available so it was not included in this clean coral sand tracer experiment. The tracer injection solution was stirred and separated into three glass bottles.

Using three dedicated MilliGat Global FIA Inc. peristaltic pumps (one for each column) operating at a rate of approximately 5 mL/min, the spiked tracer injection solution was dripped on to the top of each column and drained under gravity. The application lasted for 1 hour, by which time the equivalent of 35 mm head of spiked effluent had been applied to each column. The cumulative volume of solution drained from the base of each sand column was collected and analysed in triplicate for its microbial content. The experiment was operated at an ambient room temperature of  $20 \pm 2^\circ\text{C}$ . During the experiment the tracer injection solution, columns and drainage were all kept in the dark (tin foil) to avoid any UV inactivation of microorganisms, and prevent growth of algae on surface of the glass or sand as the algae would potentially change transport rates.

The pre-experiment drainage results showed no background levels of microorganisms (*E. coli* J6-2; *E. faecalis*; MS2 phage, adenovirus, echovirus and rotavirus) within the columns (data not shown).

The results from the clean coral sand experiment shown in Figure 4.3 reveal that the concentration of all the microorganisms which were spiked into the AGW and applied onto the columns were approximately  $1 \times 10^6 - 10^7$  counts or genome copies / mL. The cumulative drainage recovery from the unsaturated columns after the application of the microorganism spiked AGW ranged between 88 and 90%. The standard error presented in Figure 4.3 was small for all sample replicates and columns which is why the results are presented as a mean for all three columns.



**Figure 4.3.:** The mean concentrations of the microorganisms in columns A, B and C during the clean coral sand experiment in the influent (applied to the columns) and effluent (drained from the columns) with standard error bars. ND denotes not detected.

Effluent enumeration results in Figure 4.3 show that none of the indicator or pathogenic microorganisms were present in the column drainage. It is surmised that this result was due to the application of the microorganism spiked AGW for only 1 hour which simulated unsaturated conditions and resulted in the microorganisms being most likely physically retained within the coral sand packed columns. If the application of the microorganism spiked AGW had occurred for a longer period, or if the column conditions were closer to saturation, then the microorganisms may have been detected in the effluent drained from the columns. This result highlights the importance of managing the dosing of a septic tank effluent disposal field to maintain a low flow application rate and unsaturated conditions. These conditions are most likely to ensure that microorganisms found in domestic effluent have the greatest opportunity to be retained within coral sand through various microbial removal mechanisms such as physical straining, attachment/adsorption, die-off, and/or predation.

Table 4.2 presents the relative difference between the influent (microbial tracer solution applied to the columns) and effluent (drainage) as effective Log Removal Values (LRVs) for the clean coral sand tracer experiment. The LRVs for the various microorganisms are reported here because LRVs are an industry standard which is used to determine the log removal which can be expected by a treatment process [LeChevallier and Au, 2004]. The results showed significant and consistent log removal, between the values of 5.9 and 6.6, occurred for all microbiological indicator organisms (*E. coli* J6-2, *E. faecalis* and MS2 phage). A greater range of LRVs were calculated for the viral pathogens, adenovirus (2.1 - 6.5 LRV) and echovirus (2.6 - 7.7 LRV) (Table 4.2). The variance attributed mainly to adenovirus and echovirus measurements made on column C. The variance in LRV provided by column C is unexplained but could be attributed to a preferential pathway within the coral sand column apparatus but there was no visible evidence of this. The LRV range for rotavirus from all columns however was consistently high (6.8 - 7.1). Norovirus was not included in the clean coral sand experiment as it was not available at the time of the experiment.

Even though the results indicate removals on average of greater than 5 LRV for all tracers, the observed microbial counts (by cell culture and PCR detection) within the effluent drained from the columns are considered to provide a more accurate analysis of the removal efficiency of coral sand. The LRV simply informs the operator that x log can be expected to be achieved by a treatment process. It does not inform the operator on the disinfection (i.e. inactivation of a pathogen) [LeChevallier and Au, 2004]. To illustrate the LRV results from the clean coral sand experiment a disposal field depth of 400 mm (as simulated in this experiment) which achieved a removal efficiency of over 5-log removal values would equate to a target microorganism reduction of 99.999% and would be sufficient to remove at least  $1 \times 10^5$  microorganisms / g faeces.

#### **4.4.2. Simulated groundwater application experiment**

Approximately 24 hours after the clean coral sand tracer experiment, AGW was applied to each column to determine if it would mobilise any of the microorganisms that had been applied to the columns. This was done to simulate a coral sand disposal bed interacting with groundwater or coming into contact with a groundwater source. The application of AGW was dripped onto the top of

#### 4.4 Clean coral sand experiments

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	<i>E. coli</i> J6-2	<i>E.</i> <i>faecalis</i>	MS2 phage	Adenovirus	Echovirus	Rotavirus	Norovirus
Column A	6.6	6.4	5.9	6.4	7.7	7.0	-
Column B	6.6	6.3	5.9	6.5	7.7	7.1	-
Column C	6.6	6.3	5.9	2.1	2.6	6.8	-
Average LRV	6.6	6.3	5.9	5.0	6.0	7.0	-

**Table 4.2.:** Calculated Log Removal Values (LRVs) for the microorganisms drained from the columns during the clean coral sand experiment. Norovirus was not available for the clean coral sand experiment.

each column at a rate of approximately 5 mL/min and drained under gravity. The application lasted for 1 hour, with the equivalent of 35 mm of head applied to each column. This application rate was the same as the previous clean coral sand tracer experiment. The cumulative drainage from the base of each column was collected and analysed in triplicate for its microbial content. The application of the AGW at the above mentioned rate however was not realistic enough to simulate a disposal bed interacting with groundwater. If groundwater were to truly interact with a disposal bed then saturated conditions should have been applied to the columns. This scenario was however was not possible due to the free draining column apparatus that was employed. This groundwater application experiment did however manage to investigate the potential effects on the microbial removal within a coral sand disposal field that comes into contact with a groundwater source (i.e. groundwater which has been used within a household for cooking or cleaning and then enters the septic tank).

The results from the simulated groundwater application revealed concentrations of microorganisms drained from all three columns that were too numerous to count. The bacterial indicators (*E. coli* J6-2 and *E. faecalis*) and viral indicator (MS2 phage) were all too numerous to count at a 1 mL serial dilution ( $> 10,000$  cfu or pfu / mL). The eluted samples were not analysed for the pathogenic viruses (adenovirus, echovirus and rotavirus) due to the limited resources available for this research.

The mobilisation of *E. coli* J6-2, *E. faecalis* and MS2 phage due to the application of AGW indicates that if groundwater were to interact with a septic tank effluent

disposal field then bacteria and viruses could be mobilised to the underlying groundwater lens.

#### 4.4.3. Clean coral sand column destructive sampling experiment

Following the simulated groundwater application experiment and within 24 hours of the clean coral sand tracer experiment, the sand columns were destructively sampled at a discrete depth interval of 0 - 50 mm. The microorganisms (*E. coli* J6-2; *E. faecalis*; MS2 phage, adenovirus, echovirus and rotavirus) retained in the sand packed columns were enumerated with extraction procedures following standard methods (APHA, 1998). That is, the full sample from 0 - 50 mm, from each of the columns, were individually placed into glass beakers and mixed thoroughly using a stainless steel spoon. In triplicate 25 g (wet weight) samples of sand were sub-sampled from each column and suspended in 250 mL of a pH 8 saline solution and shaken vigorously by hand for 1 minute. Microbial counts were performed in triplicate on 1 mL of the wash extract and calculated as counts per gram dry weight of sand.

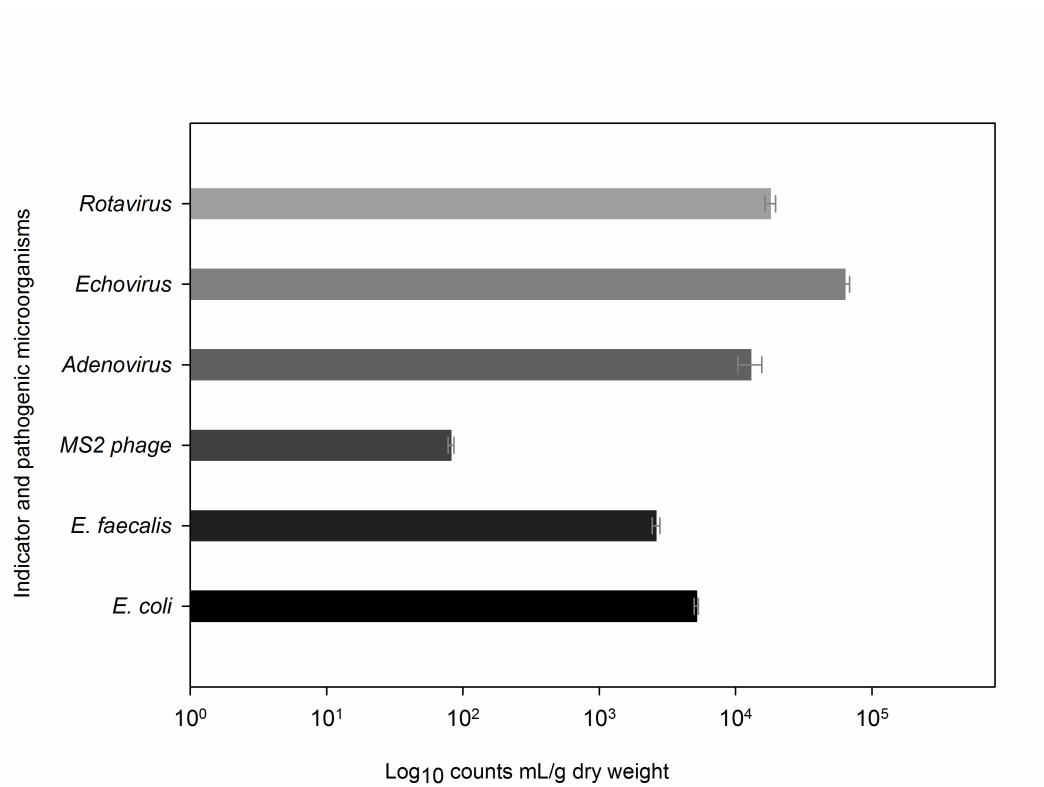
Enumeration results shown in Figure 4.4 indicate the proportion of microorganisms which were retained within the top 50 mm of coral sand from each column. *E. coli* J6-2 and *E. faecalis* were found in concentrations of approximately 4,320 – 5,820 and 1,740 – 3,420 counts mL/g dry weight. The viral indicator MS2 phage was found in concentrations between 96 – 100 counts mL/g dry weight while the viral pathogens (adenovirus, echovirus and rotavirus) were found in concentrations of between 7,800 – 72,200 counts mL/g dry weight. The standard error presented in Figure 4.4 was small for all sample replicates and columns which is why the results are presented as a mean for all three columns. The remaining sections of the columns (50 – 400 mm) were not analysed for their microbial content due to limited resources available for this research.

Compared to the concentration of microorganisms which were applied to the columns ( $1 \times 10^6$  -  $10^7$  counts or genome copies / mL) the percentage which were retained within the top 50 mm of each column was approximately 0.1 - 10% with the exception of MS2 phage which was <0.1%. Since the lower layers of the columns could not being analysed for their microbial content no conclusions as to the significance of the microorganisms which were found in the top 50 mm of the columns could be made.



A limitation of the technique that was used to extract the microorganisms from the coral sand is the use of hand shaking for only 1 minute. Wild et al. [2006] highlights the need for homogenisation, ultrasonication and centrifugation to detach and enumerate microorganisms in calcium carbonate sediments. This is because microorganisms may be deep within the pores of the calcium carbonate matrix and be difficult to detach [Wild et al., 2006]. The enumeration results for the microbial content of the sand are therefore likely to be an under estimate.

Another limitation of this research involves the enumeration of viral pathogens by PCR techniques. PCR is a highly versatile and sensitive technique for the detection of DNA within specific organisms and can detect very small numbers of microbes within a sample [Mara and Horan, 2003]. The only limitation in using PCR technology to detect viruses is that it detects the total number of virus particles in a sample and gives no indication of the proportion of virus particles that are potentially infectious or any indication of their viability (i.e. if they are whole organisms or fragments) hence the technique can overestimate the risk of exposure [Mara and Horan, 2003]. The results presented here therefore represent a conservative scenario with the viable viruses most likely to be less than the concentrations which were retained within the top 50 mm of coral sand.



**Figure 4.4.:** The mean concentrations of microorganisms contained within the top 50 mm of columns A, B and C after the clean coral sand experiment with standard error bars.

## **5. Microbial removal in conditioned coral sand**

After determining the physio-chemical properties of coral sand and the ability of clean coral sand to remove microorganisms the next step was to examine the ability of domestic effluent contaminated coral sand to remove microorganisms. The following chapter describes the materials and methods employed in the unsaturated conditioned coral sand packed laboratory column experiments with the results presented and discussed. Conditioned coral sand describes the “aging” of the coral sand after application of domestic effluent over time.

### **5.1. Laboratory column apparatus**

The same laboratory column apparatus that were used in the clean coral sand experiments (Section 4.1) were used in the conditioned coral sand experiments. The same limitations that were discussed in the clean coral sand tracer experiment, such as coral sand particle size and porosity (Section 4.1) similarly apply to the conditioned coral sand tracer experiments. Re-packing of the columns after the clean coral sand experiments resulted in the dry bulk density and total porosity values given in Table 5.1 which are comparable to the clean coral sand experiment values.

	Column A	Column B	Column C
<i>Dry Bulk Density (<math>\text{g}/\text{cm}^3</math>)</i>			
Clean coral sand experiments	1.49	1.49	1.49
Conditioned coral sand experiments	1.45	1.45	1.46
<i>Total Porosity (%)</i>			
Clean coral sand experiments	26	28	27
Conditioned coral sand experiments	27	27	27

**Table 5.1.:** The dry bulk density ( $\text{g}/\text{cm}^3$ ) and total porosity (%) results for the Bikenibeu beach coral sand packed columns during the clean and conditioned coral sand experiments.

## 5.2. Microbial properties, propagation and preparation

The same pathogenic microorganisms used in the clean coral sand experiments (Section 4.2) were used in the conditioned coral sand experiments with the exception of norovirus which was included in the suite of microorganisms applied to the columns. Norovirus was extracted from human stool samples that were diluted in Phosphate Buffered Saline (PBS), pH 7.2, then filtered through a 0.22  $\mu\text{m}$  filter, before being stored at 4°C before being added to the experiment injection solution.

## 5.3. Microbial enumeration

The same methods that were used for the pathogenic microorganism enumeration in the clean coral sand experiments (Section 4.3) were used in the conditioned coral sand experiments.

## 5.4. Conditioned coral sand experiments

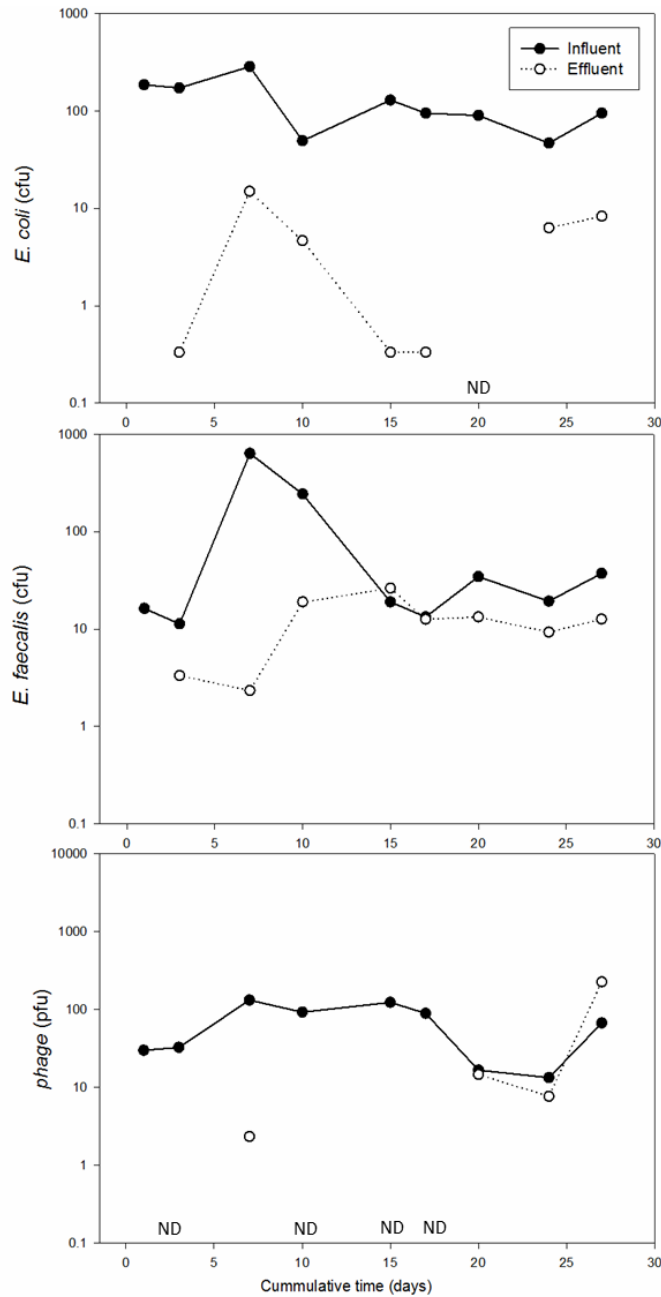
### 5.4.1. Column conditioning

It is widely reported that microbial removal rates measured in clean porous media differ from those measured in the same media that has been conditioned or

contaminated with effluent [Wall et al., 2008, Pang, 2009, Weaver et al., 2013, Sinton et al., 2010, Harvey et al., 2010]. This is due to the blocking of potential attachment sites on the surface of porous media by organic matter, which is abundant in domestic effluent, and can decrease the attachment rate over time and enhance the transport of microorganisms [Schijven et al., 2017]. The organic matter content of effluent is measured by Dissolved Organic Carbon (DOC). Harvey et al. [2010] stated that multiple studies have found DOC to enhance microbial transport but cautioned that more recent studies suggest that this is not always the case. For example Cheng et al. [2007] found that DOC had no significant influence on the transport of MS2 bacteriophage in sandy soil. Whether DOC promotes or inhibits microbial transport in porous media is complex and dependent on experimental conditions, aqueous chemistry, and the properties of the organic matter, microbes and porous media surface [Harvey et al., 2010]. The purpose of conditioning the columns with domestic effluent which is abundant in DOC, for as long as the research resources would allow (27 days), was to investigate the microbial attenuation within the coral sands in an aged (conditioned) system. It was also done to see if a *Schmutzdecke* layer would form, as previously defined in Section 2.4.1, and to observe if this layer would enhance or inhibit microbial removal.

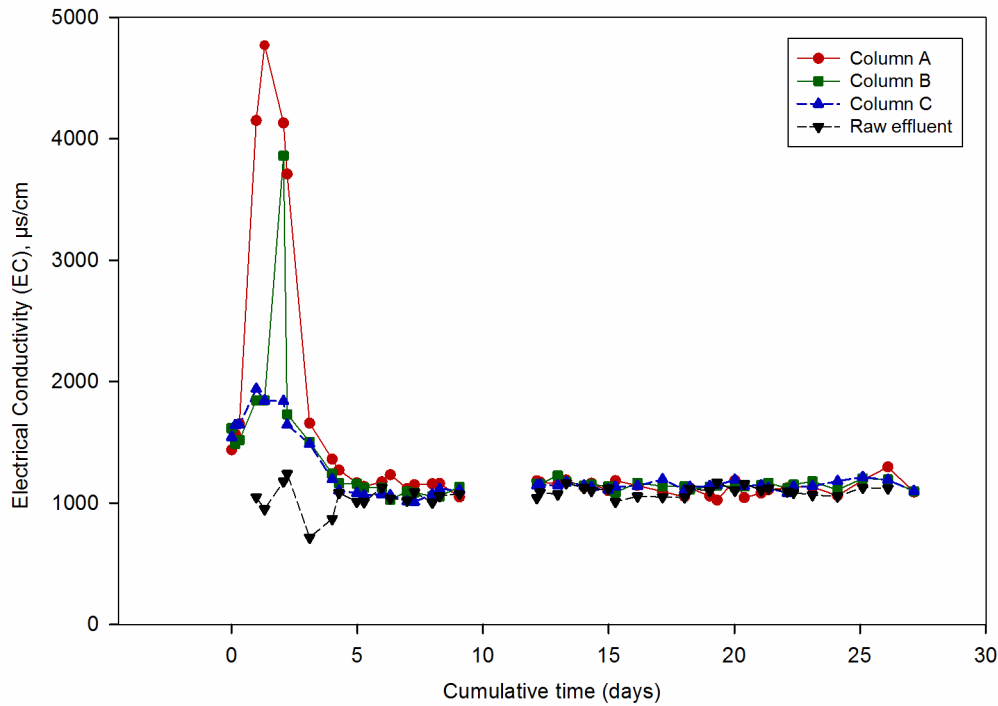
The concentrations of naturalised (found naturally in the environment) *E. coli*, *E. faecalis* and bacteriophage that were measured during the 27 days of column conditioning with domestic septic tank effluent are given in Figure 5.1. The concentrations of naturalised *E. coli*, *E. faecalis* and phage in the raw effluent that was applied to the columns (influent) were reasonably consistent throughout the 27 days of column conditioning (Figure 5.1). Some variation was most likely due to changes in variables such as septic tank useage/flow, faecal input, temperature and time of sampling. Figure 5.1 also shows that the concentrations of naturalised *E. coli*, *E. faecalis* and phage drained from the columns (effluent) were variable throughout the 27 days with several not detected results for *E. coli* and phage. When the effluent concentrations exceeded the influent concentrations this may have been a indication that the columns were maturing with many of the attachment sites potentially being occupied by the organic carbon resulting in the microorganisms exiting the columns as via the drainage. Figure 5.1 shows that influent and effluent concentrations of *E. coli* ranged from 50 – 287 cfu and 0 – 15 cfu respectively. Influent and effluent concentrations for *E. faecalis* ranged from 11

– 637 cfu and 0 – 26 cfu respectively. The influent and effluent concentrations results for bacteriophage ranged from 13 – 132 pfu and 0 – 226 pfu respectively.



**Figure 5.1.:** The mean microorganism concentrations for naturalised *E. coli*, *E. faecalis* and phage in column A, B and C's influent and effluent during the conditioning for 27 days. ND denotes not detected

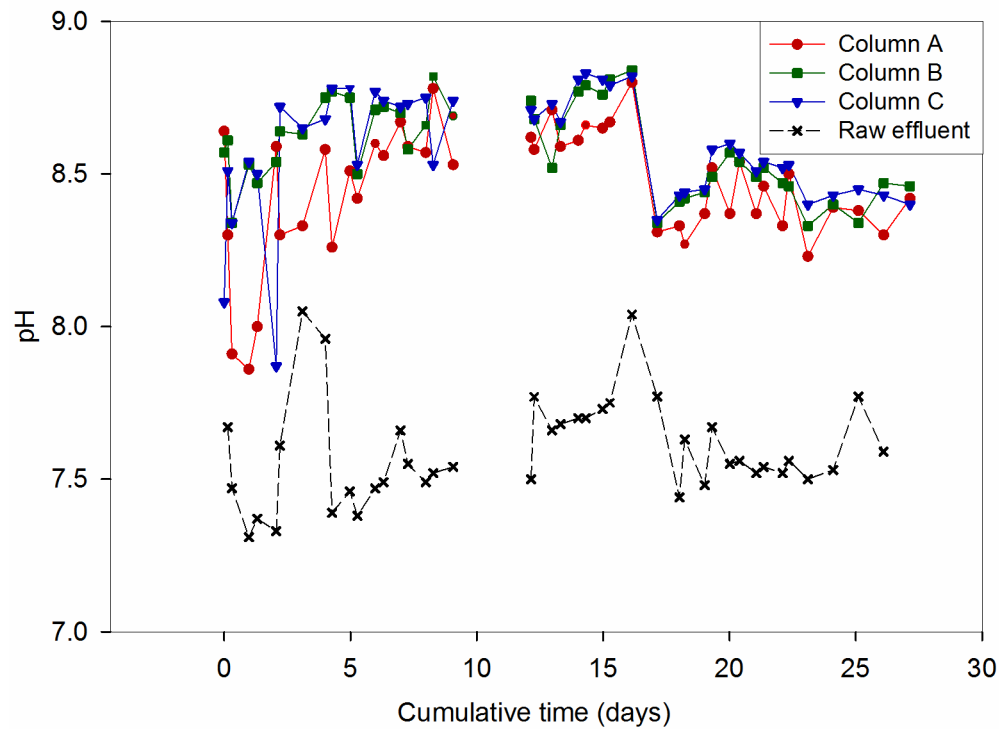
After two days of column conditioning the Electrical Conductivity (EC) of the drainage from columns A and B peaked at 4,770 and 3,860  $\mu\text{S}/\text{cm}$  respectively after which both columns stabilised and gave similar results to column C (Figure 5.2). No EC measurements were taken on days 10 - 11.



**Figure 5.2.:** Electrical Conductivity (EC) of the applied raw effluent and the drainage from columns A, B and C as monitored during the column conditioning for 27 days.

The reason for this spike in EC is unclear and could be due to a natural peak due to an ion exchange process flushing mineral ions off the coral sand. It is also unclear as to why it was only observed in columns A and B. After 4 days of conditioning the drainage from all three columns and the raw effluent that was applied to the columns all had similar EC results. On average, for the 27 days of conditioning, the EC from all three columns was 1,324  $\mu\text{S}/\text{cm}$ . The lowest recorded EC from any of the columns drained from column C, 1,009  $\mu\text{S}/\text{cm}$ .

The difference in pH between the applied raw effluent and the drainage from the three columns was approximately a pH unit throughout the column conditioning period of 27 days (Figure 5.3).



**Figure 5.3.:** pH of the applied raw effluent and the drainage from columns A, B and C as monitored during the column conditioning for 27 days.

The raw effluent that was applied to the columns was consistently a pH unit lower than the drainage from the three columns. The average (min-max) pH from all three columns was 8.54 (7.86 - 8.84). At day 17 the pH for all three columns and the raw effluent that was applied to the columns decreased abruptly by approximately half a pH unit but stabilised by the end of the column conditioning with an approximate pH unit difference remaining between the columns and the applied raw effluent. It is unclear why this abrupt change in pH occurred but could be due to changes in variables such as septic tank usage/flow, faecal input, temperature, time of sampling. No pH measurements were taken on days 10 - 11.

The domestic effluent applied to the columns was analysed for its chemical parameters two days prior to the conditioned tracer experiment on day 25 of the column conditioning period with the results given in Table 5.2. The most important chemical parameter analysed from the domestic effluent applied to the columns was the DOC measurement which was analysed as Dissolved Non-Purgeable Organic Carbon (DNPOC) with a value of 14.4 g/m<sup>3</sup>. Only one



## 5.4 Conditioned coral sand experiments

sample was analysed for DNPOC throughout the conditioning of the columns due to limited resources available for this research. Appendix A shows the column drainage percentage recovery during the coral sand column conditioning for 27 days. The average drainage recovery for the 27 days from columns A, B and C ranged consistently between 89 - 90%.

Analyte	Chemical parameters of the domestic effluent applied to the columns on day 25 of conditioning
pH	7.6
Electrical Conductivity (EC), mS/m	76.5
Total Suspended Solids, mg/L	130
Total Nitrogen, mg/L	26
Total Phosphorus, mg/L	15
Dissolved Total Biochemical Oxygen Demand (TBOD <sub>5</sub> ), g O <sub>2</sub> /m <sup>3</sup>	4
Dissolved Non-Purgeable Organic Carbon, mg/L	14.4
<i>Escherichia coli</i> , cfu / 100 mL	170,000
<i>Enterococci</i> , cfu / 100 mL	2,200

**Table 5.2.:** Chemical parameters of the domestic effluent applied to the columns at day 25 during the column conditioning.

After the 27 days of conditioning a *Schmutzdecke* layer did form as shown in Figure 5.4. The *Schmutzdecke* layer was distinguishable by a thin dark layer at the very top of each column.



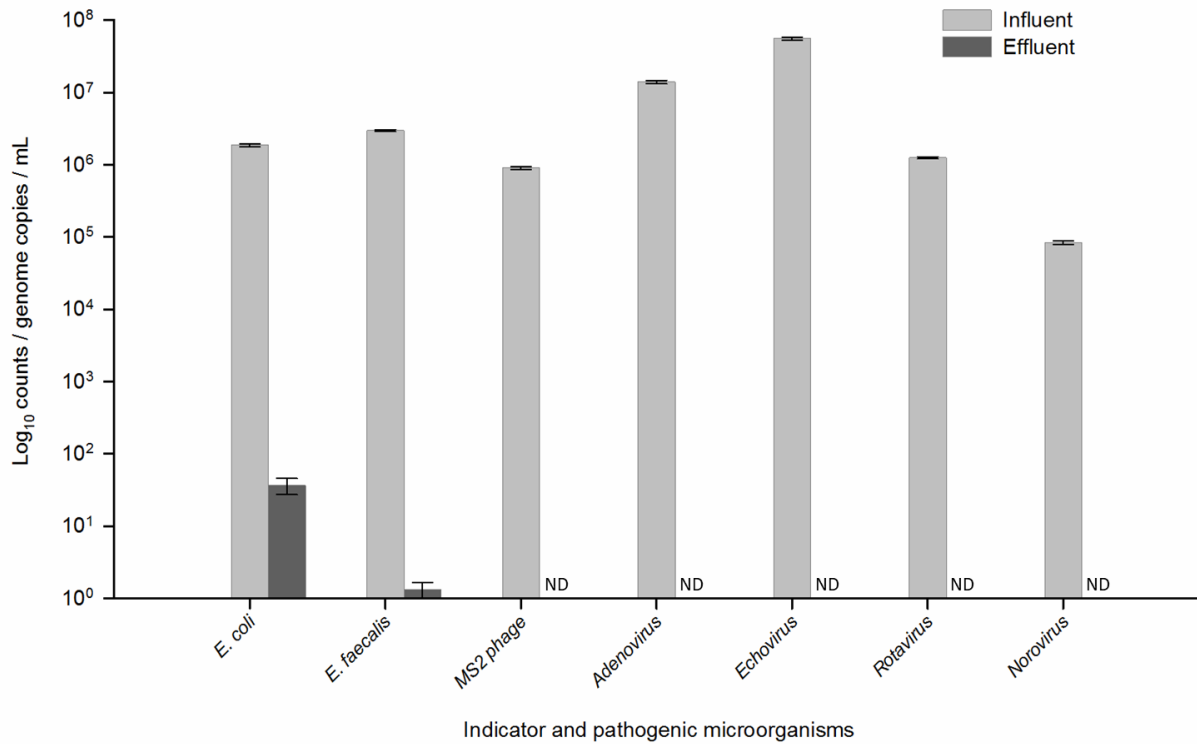
**Figure 5.4.:** Visible *Schmutzdecke* layer (dark brown) on the top of columns A, B and C at day 27 of the column conditioning.

### 5.4.2. Conditioned coral sand tracer experiment

The same methods as described in Section 4.4.1 were used to apply the microorganisms to the columns. The only exception being that instead of the AGW as the base for the microorganism spiked injection solution, the same source of domestic effluent, which was used to condition the columns for 27 days, was used as the injection solution base to which the microorganisms were spiked. For the conditioned coral sand experiments norovirus was available.

Similar to the clean coral sand experiments Figure 5.5 shows that, with the exception of norovirus, the concentration of all the microorganisms which were spiked into the domestic effluent and applied to the columns were approximately  $1 \times 10^6 - 10^7$  counts or genome copies / mL. Due to availability issues a lower input concentration of norovirus was applied to the columns of approximately  $1 \times 10^4$  counts or genome copies / mL. The cumulative drainage recovery from the unsaturated columns after the application of the microbial tracers that were mixed into the AGW ranged between 86 and 90%. The previous clean coral sand tracer experiments drainage ranged from between 88 and 90% therefore the drainage does not appear to have been impacted by the column conditioning.

The indicator bacteria results show that *E. coli* J6-2 and to a lesser extent *E. faecalis*, which were the largest organisms tested, were the most mobile during the conditioned experiment (Figure 5.5). With an average concentration of 36 cfu / mL, *E. coli* J6-2 was found in greater concentrations in the drainage than *E. faecalis* (1 cfu / mL). The indicator bacteria results are in agreement with those of Gupta et al. [2009], Bales et al. [1995] and Aronino et al. [2009] which all found that larger bacterial cells showed greater transport (i.e. less removal) than smaller cells such as viruses in silica sand. The bacterial indicators, especially *E. coli* J6-2, appear to have been transported within the coral sand by the initial pulse flow of the injection solution. The fact that *E. coli* J6-2 are motile bacteria which are propelled by their flagella may have been a contributing factor as to why they were enumerated in the effluent which drained from the columns. Tufenkji [2007] identifies that well-controlled studies that have investigated the role of microbial motility in porous media are scarce, meaning there are few studies to support this theory. In the absence of any conservative tracer such as bromide, in this experiment *E. coli* J6-2 can be considered to be the most conservative microbial tracer.



**Figure 5.5.:** The mean concentrations of the microorganisms in columns A, B and C during the conditioned coral sand experiment in the influent (applied to the columns) and effluent (drained from the columns) with error bars. ND denotes not-detected.

In contrast to the larger indicator bacteria, the smaller viral indicator (MS2 phage) and pathogenic viruses (adenovirus, echovirus, norovirus and rotavirus) were the least mobile (i.e. greatest removal) of all the microbial tracers (Figure 5.5). The size of indicator and pathogenic viruses employed in this study were all between 0.028 – 0.09  $\mu\text{m}$  according to Pang [2009]. This indicates that physical straining was not a dominant removal mechanism and adsorption may have been more significant. These results are in contrast to the saturated experiments detailed in Burberry et al. [2015]. Burberry et al. [2015] found that under saturated conditions the Bonriki freshwater reserve coral sand was more effective at attenuating *E. coli* J6-2 than MS2 phage.

According to Farkas et al. [2014] electrostatic and hydrophobic interactions largely control the process of absorption. A graph provided in Pang [2009] show that all the indicator and pathogenic viruses used in this study had negative surface charges of between approximately -20 to -40 mV at pH 8. This is supported by Aronino et al. [2009] who found that bacteriophages tended to be more negatively charged at higher pH values. Aronino et al. [2009] found for bacteriophages at pH 4 the average zeta potential was -10 mV, but decreased to -30 mV at pH 8. Further investigation is required but the zeta potential results obtained for the Bikenibeu beach coral sand at pH 8, were less negative than the viral tracers (-7.4 mV, with a Standard Error of 0.87), and indicate that electrostatic interactions between the viruses and the coral sand may have been a contributing factor in their attraction (adsorption) to each other and their subsequent retention. Torkzaban et al. [2013] observed that viruses strongly attach to mineral surfaces in the presence of  $\text{Ca}^{2+}$  potential indicating that coral sand may have a reactive surface. Gao and Saiers [2006] state that porous media which are more reactive than silica sand, which may include carbonaceous coral sand, will be capable of binding greater quantities of microorganisms [Gao and Saiers, 2006]. It is important to note however that retention does not necessarily mean inactivation. This is particularly relevant when the process of reverse adsorption (desorption) occurs due to changes in ionic strength as a result of rainfall [O’Luanaigh et al., 2012].

The indicator and pathogenic viruses during the conditioned tracer experiment behaved the same as each other in that MS2 phage, adenovirus, echovirus, norovirus and rotavirus were not present in the drainage from any of the columns. This indicates that directly after column conditioning MS2 phage appears to be a good surrogate for the pathogenic viruses. Schijven et al. [2003] observed that absorption was the most significant factor in the elimination and retention of MS2 phage [Schijven et al., 2003]. Absorption therefore could be the dominant removal mechanism for the indicator and pathogenic viruses.

As previously mentioned in Section 5.4.1, the addition of DOC to the columns, in the form of domestic effluent, may have influenced the microbial attachment of microorganisms and enhanced their transport but this is not always the case. Harvey et al. [2010] states that the issue of whether DOC promotes or inhibits microbial transport in granular media is complex. The experimental conditions,

aqueous chemistry, the organic matter, microorganisms and grain surfaces can all influence microbial attachment [Harvey et al., 2010].

Out of all the microorganisms, the largest organisms, *E. coli* J6-2 and *E. faecalis*, were observed within the effluent drained from the columns. This indicates that their attachment and transport may have been negatively influenced by the occupation of DOC on potential attachment sites on the surface of the coral sand as a result of the column conditioning. The occupation of DOC on the surface of the coral sand may have blocked the indicator bacteria's opportunities for adsorption. The smaller viral indicator (MS2 phage) and pathogenic viruses (adenovirus, echovirus, norovirus and rotavirus), however, appeared to be positively influenced by the presence of DOC in the form of the domestic effluent which was applied to the columns during the conditioning period. A study by Schijven and Hassanizadeh [2000] found that organic carbon bound to porous media created additional binding sites and increased the rate of virus absorption.

Determining the mechanisms by which the microorganisms were removed from the coral sand was beyond the scope of this study. Tufenkji [2007] states that physical straining is not considered an important removal mechanism when the bacteria to grain size diameter ratio is small ( $< 0.05$ ). In this experiment the bacteria to grain diameter ratio was approximately 0.006 and 0.0012 for *E. coli* J6-2 and *E. faecalis* respectively. This finding suggests that adsorption of the viral indicator and pathogens onto the surface of the coral sand may have been a significant removal mechanism. According to Bales et al. [1991] batch tests would need to be confirm if adsorption is a significant microbial removal mechanism within coral sands but was beyond the scope and resources of this research.

The limitation of the above mentioned results are that the application of domestic effluent spiked with microorganisms was a single application and not followed up with continual loading of the system for a period of time. If the system had been loaded with high concentrations of microorganisms for a period of time then perhaps conclusions with regards to the dominant removal mechanisms would have become evident. This scenario was not possible however due to limited resources available for this research.

Table 5.3 presents the relative difference between the influent and effluent (drainage) as effective LRVs for the conditioned coral sand tracer experiment. The results show a significant and consistent log removal occurring for all

microbiological indicator organisms (*E. coli* J6-2, *E. faecalis* and MS2 phage) ranging from 3.9 – 6.4 LRV. Good replicability was observed across all columns. Consistently high removal efficiencies were observed for the viral pathogens, namely adenovirus, echovirus, rotavirus and norovirus (Table 5.3). Norovirus appears to have a lower LRV but this was an artefact, related to the lower input concentration of norovirus (approx.  $1 \times 10^4$  cfu / mL) available at the time of the experiment comparative to the other microbial tracers. The results from the conditioned coral sand experiment indicate on average removals greater than 4 LRV for all microorganisms which was slightly less than the clean coral sand experiment. To illustrate the LRV results from the conditioned coral sand experiment a disposal field depth of 400 mm (as simulated in this experiment) which achieved a removal efficiency of over 4-log removal values would equate to a target microorganism reduction of 99.99% and would be sufficient to remove at least  $1 \times 10^4$  microorganisms / g faeces.

	<i>E. coli</i> J6-2	<i>E.</i> <i>faecalis</i>	MS2 phage	Adenovirus	Echovirus	Rotavirus	Norovirus
Column A	4.2	3.9	6.0	7.2	7.0	7.0	4.9
Column B	4.0	4.9	5.9	7.1	7.1	7.1	4.9
Column C	3.9	6.4	5.9	7.1	7.1	7.1	4.8
Average LRV	4.0	5.1	5.9	7.1	7.0	7.0	4.9

**Table 5.3.:** Calculated Log Removal Values (LRVs) for the microorganisms drained from the columns during the conditioned coral sand experiment.

Table 5.4 provides a comparison of microbial removal studies in unsaturated laboratory disturbed porous media filled columns [Hijnen et al., 2005, Seeger et al., 2016]. The LRVs achieved in this research are higher than any of the materials mentioned in Table 5.4. These studies used comparable methods and apparatus to this research and so illustrate the effectiveness and potential of coral sand to attenuate microorganisms.

Reference	Porous media	Column dimensions	Microorganisms	Log <sub>10</sub> reduction
Hijnen et al. [2005]	Dune sand	90 mm diameter 500 mm long	MS2 phage	2.20 - 3.30
Hijnen et al. [2005]	Coarse and fine gravel with sand	90 mm diameter 500 mm long	<i>E. coli</i>	4.10 - 4.80
Seeger et al. [2016]	Quartz sand	200 mm diameter 1300 mm long	<i>E. coli</i> & <i>Enterococci</i>	$\leq 4.7$ & $\leq 2.4$

**Table 5.4.:** Comparison of Log<sub>10</sub> microbial removal in unsaturated disturbed laboratory porous media filled columns.

### 5.4.3. Simulated heavy rainfall experiment

Within 24 hours of the conditioned coral sand experiment a heavy rainfall event was simulated on the columns. Future model predictions from the Pacific Climate Change Science Program and the Kiribati Meteorology Service show that extreme rainfall days are likely to occur more often in Kiribati [PCCSP, 2011]. The aim of simulating a heavy rainfall event was to observe the response of the microorganisms within the coral sand due to a reduction in ionic strength provided by the rainwater. Ionic strength is the measure of the concentration of dissolved chemical ions in solution [Metcalf et al., 2003]. It is well documented that a reduction in ionic strength can promote mobilisation of microorganisms by increasing the repulsive forces between attached colloids and mineral grains [Gao et al., 2004]

Monthly totals of rainfall are only available for South Tarawa. In the wettest month (January) there are on average 20 days of rainfall with the highest recorded mean rainfall of 270 mm [SPC, 2014]. The evening prior to the experiment rain from Christchurch (New Zealand) was collected over a period of 12 hours and based on the highest monthly rainfall mean 120 mL was applied to each column over a period of 5 minutes. The rainwater was analysed for background levels of the target microorganisms with none being found. After the application of the rainwater to the top of the columns the cumulative drainage from the base of each column was collected and analysed in triplicate for its microbial content (*E. coli*

J6-2; *E. faecalis*; MS2 phage, adenovirus, echovirus, norovirus and rotavirus). The cumulative drainage recovery from the unsaturated columns after the application of the rainfall event ranged between 67% and 76%. In comparison to the clean and conditioned coral sand tracer experiments, which both had higher drainage recovery of between 86 - 90%, the reduced drainage recovery after the simulated heavy rainfall experiment indicates that prior to the simulated heavy rainfall experiment the columns may have experienced some drying out.

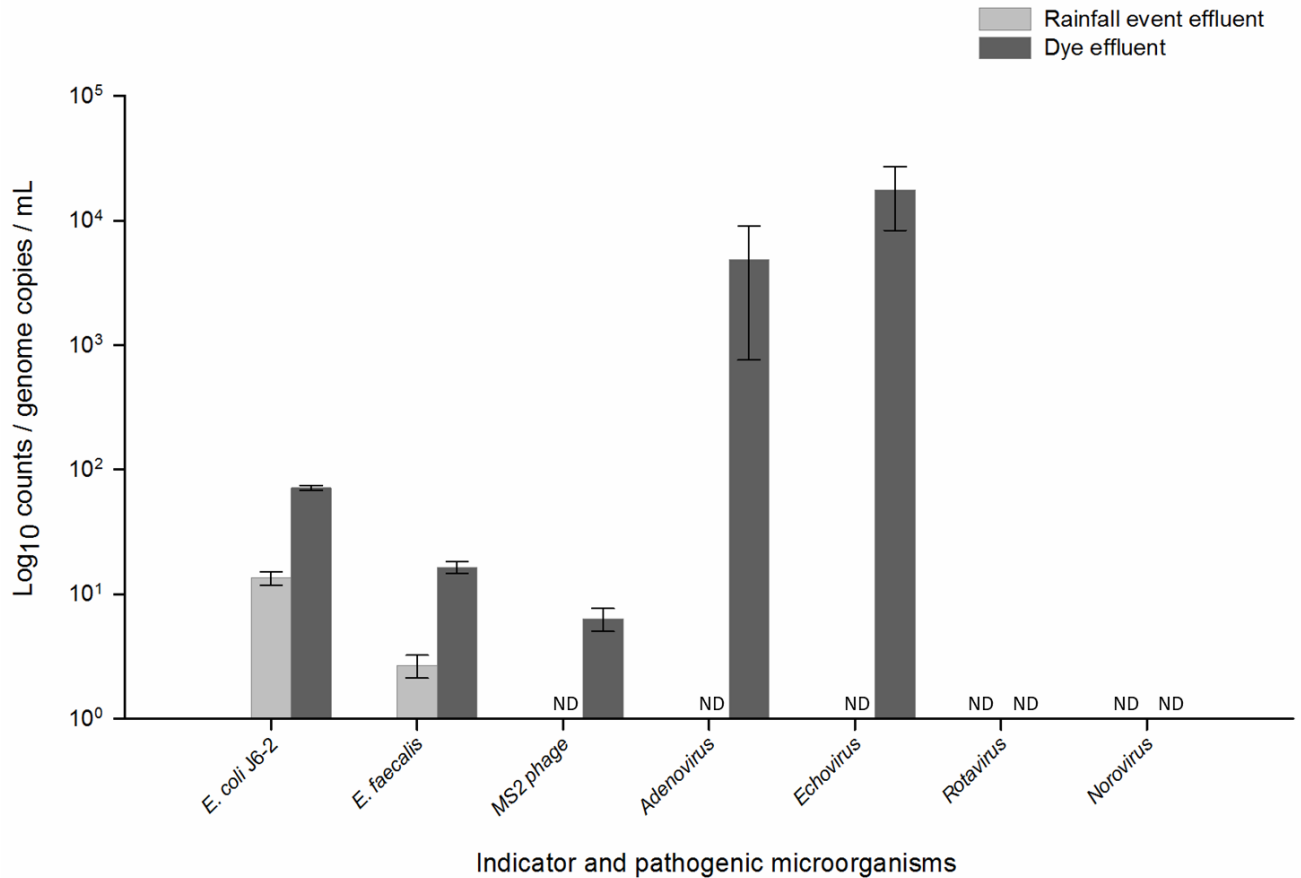
The simulated rainfall event resulted in the transport of low numbers of bacterial indicator organisms through the columns; with averages of 13 cfu per mL and 3 cfu per mL for *E. coli* J6-2 and *E. faecalis* respectively (Figure 5.6). No evidence was found of MS2 phage, adenovirus, echovirus, norovirus or rotavirus in the drained effluent. This finding replicates the results from the previous day, during the conditioned coral sand tracer experiment, in that only *E. coli* J6-2 and *E. faecalis* were transported through the coral sand columns.

#### **5.4.4. Dye tracer experiment**

Within 24 hours of the simulated heavy rainfall event green food dye mixed with the same rainwater used in Section 5.5.3 was applied to each column as a visual tracer. The aim of applying a dye tracer to the columns was to visually assess if any preferential flow pathways or plug flow existed within the columns.

The green food dye was applied to the columns at a rate of 5 mL / min. The application lasted for 1 hour, with the equivalent of 35 mm of head applied to each column which is the same rate as the clean and conditioned coral sand tracer experiments. The cumulative volume of solution drained from the base of each sand column was collected and analysed in triplicate for its microbial content (*E. coli* J6-2; *E. faecalis*; MS2 phage, adenovirus, echovirus, norovirus and rotavirus).

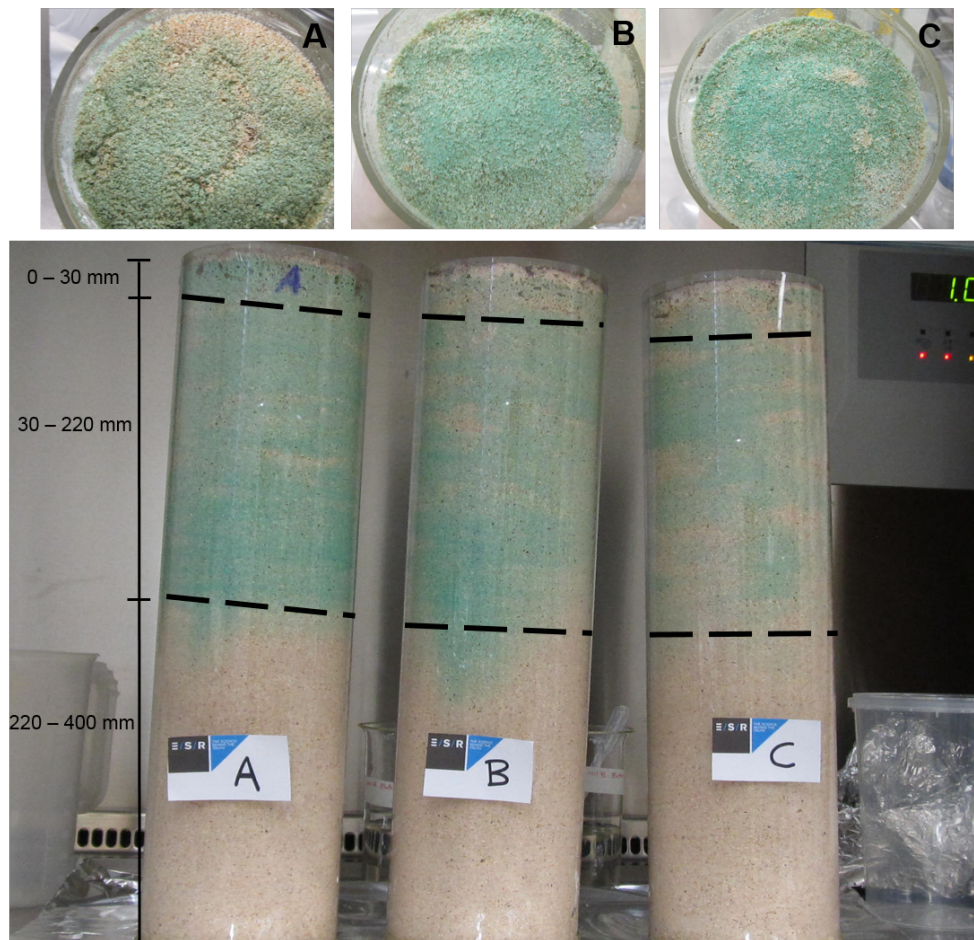




**Figure 5.6.:** The mean concentrations of the indicator and pathogenic microorganisms drained from columns A, B and C during the simulated heavy rainfall experiment and the dye experiment with standard error bars. ND denotes not-detected.

From the dye tracer experiment we deduce that the coral sand was uniformly consolidated, since no obvious preferential flow pathways were observed, as might have been witnessed through mottled dye effects in the sand pack (Figure 5.7). The dye also penetrated down to a depth of 220 mm within all three columns after which point no green dye was evident in the coral sand. It is interesting to note that when the dye solution was applied to the columns a significant mobilisation of *E. coli* J6-2, *E. faecalis*, MS2 phage, adenovirus and echovirus were observed (Figure 5.6). Rotavirus and norovirus were not however present in the dye effluent. The reasons for their absence are unknown as rotavirus and norovirus were neither

the largest or smallest of the viral pathogens. The surface charge properties of rotavirus and norovirus however may have influenced their absorption onto the coral sands but investigating this was beyond the scope of this research.



**Figure 5.7.:** Dye tracer test results illustrating the relatively uniform path of solute transport through the columns. The top three photos were taken looking down into the columns A, B and C at a depth of 30 mm and reveal no significant evidence of preferential flow as would otherwise be observed as mottling.

Even though 24 hours apart, the second application of low ionic strength rainwater to the columns during the dye tracer experiment may have caused the mobilisation of *E. coli* J6-2, *E. faecalis*, MS2 phage, adenovirus and echovirus. This result highlights the need to manage a domestic effluent septic tank disposal field to avoid saturated conditions, as presented by heavy rainfall events, and create as much of a buffer between the effluent application point and the underlying groundwater lens to maximise the opportunity for microbial removal.

As already mentioned in Section 4.4.3 due to the analysis methods employed for the detection of pathogenic viruses (i.e. RT-PCR) no indication of their viability could be made. By determining their viability it could have been possible to eliminate various removal mechanisms such as predation and die-off. It is important to note that determining if the pathogenic viruses were retained and still viable or if they were instead inactivated would have filled a significant knowledge gap in terms of the microbial removal properties of coral sand. Since MS2 phage was enumerated using cell culture methods and was found to be a good surrogate for the pathogenic viruses throughout the experiments presented in this research it can be assumed that the pathogenic viruses were still viable and capable of infection.

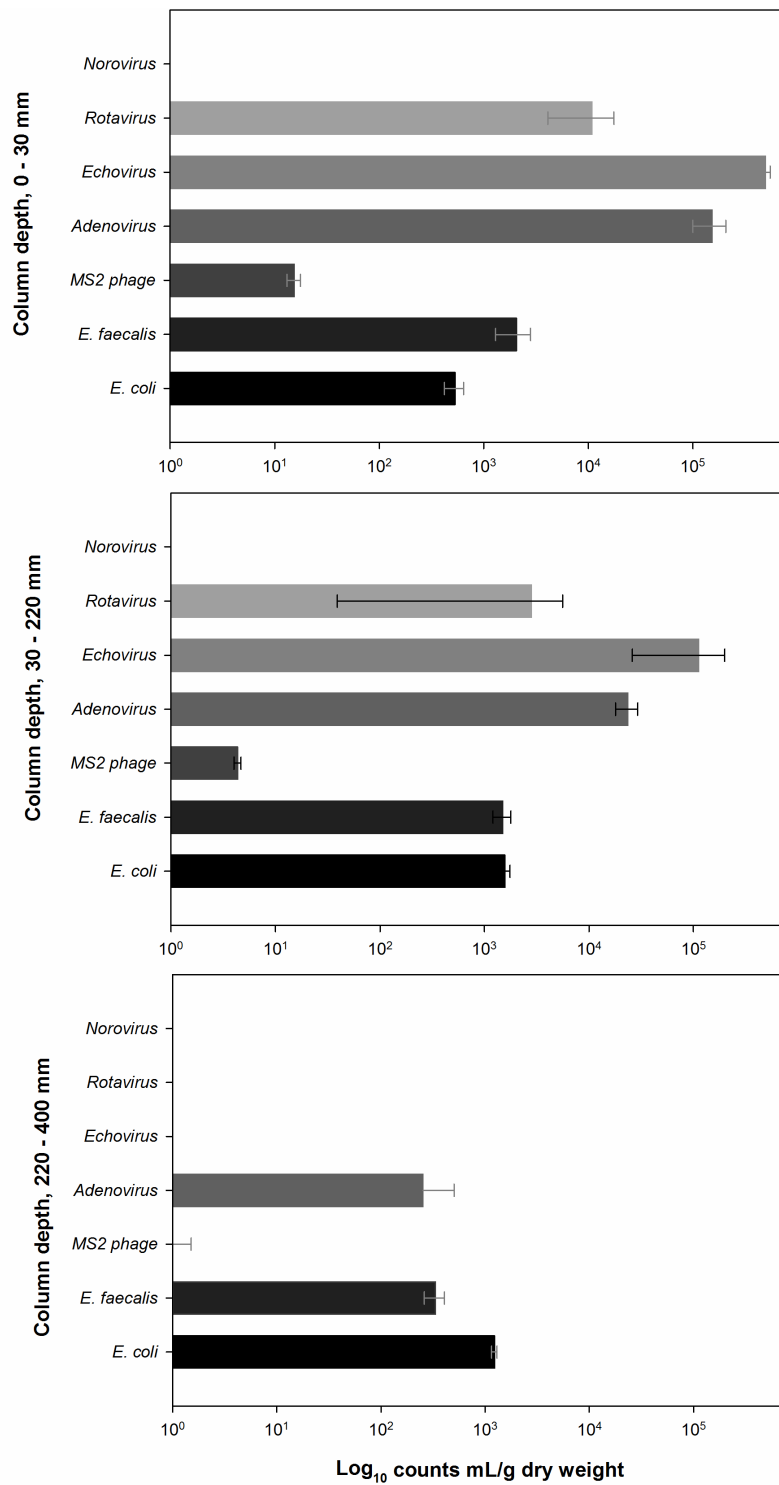
The significant mobilisation of *E. coli* J6-2, *E. faecalis*, MS2 phage, adenovirus and echovirus observed after the dye tracer experiment (and second application of rainwater) indicates that the lower ionic strength rain water could have promoted the detachment of absorbed indicator and pathogenic microorganisms.

### **5.4.5. Conditioned coral sand column destructive sampling experiment**

From the previous clean coral sand tracer experiment it was decided to increase the sampling of the sand within the columns. This was done to study the entire length of the columns and to investigate possible removal mechanisms.

On the same day as the dye tracer experiment the coral sand columns were destructively sampled at discrete depth intervals of 0 - 30 mm, 30 - 220 mm, and 220 - 400 mm. As the sand was removed from the columns photographs at the same level in each column were taken to visually capture the transport of the green dye throughout the columns and to give an indication of the presence of any potential microbial pathways. Target organisms (*E. coli* J6-2; *E. faecalis*; MS2 bacteriophage, adenovirus, echovirus, norovirus and rotavirus) were sampled via the same extraction methods as described in Section 4.4.3.

Enumeration results shown in Figure 5.8 illustrate that a proportion of microorganisms were retained within the top 30 mm of coral sand from columns A, B and C with the concentration of microorganisms decreasing with depth. At each depth interval norovirus was not detected.



**Figure 5.8.:** The mean concentrations of microorganisms found in the top 30 mm, 30 - 220 mm and 220 - 400 mm of columns A, B and C after the conditioned coral sand experiment with standard error bars included. ND denotes not-detected.

From 0 – 30 mm within each column is where a visible *Schmutzdecke* was identified (Figure 5.4). Within this interval the highest average concentrations of pathogenic viruses were detected for echovirus (496,000 counts mL / g dry weight) followed by adenovirus (230,000 counts mL/g dry weight) and rotavirus (10,841 counts mL / g dry weight). The indicator microorganisms were 2 – 3 orders of magnitude lower than the pathogenic microorganisms with *E. faecalis* (2,050 counts mL / g dry weight), followed by *E. coli* J6-2 (530 counts mL / g dry weight) and MS2 phage (15 counts mL / g dry weight).

Within the column depth interval 30 – 220 mm the pathogenic viruses detected followed a similar concentration pattern to the 0 – 30 mm depth interval. The highest average concentrations of pathogenic viruses were detected for echovirus (112,513 counts / mL / g dry weight), followed by adenovirus (23,625 counts / mL / g dry weight) and rotavirus (2,819 counts / mL / g dry weight). As the error bars show there was a large amount of variability in the rotavirus concentrations between the three columns. Similar to the 0 – 30 mm depth interval the indicator microorganisms were 2 – 4 orders of magnitude lower than the pathogenic microorganisms with *E. coli* J6-2 (1,570 counts / mL / g dry weight), followed by *E. faecalis* (1,500 counts / mL / g dry weight) and MS2 phage (4 counts / mL / g dry weight).

Within the 220 – 400 mm column depth interval norovirus, rotavirus and echovirus were not detected and only 1 count mL/g dry weight for MS2 phage. The highest average microbial concentrations were detected for *E. coli* J6-2 (1,230 counts / mL / g dry weight) followed by *E. faecalis* (333 counts / mL / g dry weight) and adenovirus (252 counts / mL / g dry weight).

As previously mentioned in Section 2.4.1 there are implications for the presence of a biological layer such as a *Schmutzdecke* which is closely related to the DOC content of the applied effluent. Zhuang and Jin [2003] observed that the presence of organic matter reduced virus attachment and in turn facilitated the transport of viruses by either providing additional negative charges, covering positively charged sites or competing with viruses for attachment sites. These processes are termed hydrophilic blocking or electrostatic repulsion which facilitates the transport of viruses [Zhuang and Jin, 2003]. This may have been why adenovirus and a small amount of MS2 phage were present in the lower section (220 - 400 mm) of the columns.

As with the previous experiments presented in this research the greatest microbial transport was observed for *E. coli* J6-2 and *E. faecalis*. In every layer of the columns *E. coli* J6-2 and *E. faecalis* were successfully enumerated within the coral sand indicating that straining, adsorption, die-off, and/or predation were not significant retention mechanisms. As previously mentioned in Section 5.4.4 because MS2 phage was considered to be a good surrogate for the pathogenic viruses within coral sand for this research it can be assumed that the pathogenic viruses found within each of the column layers, were still viable and capable of infection.

The non-detection of norovirus within the effluent and the coral sand is unexplained and could be attributed to the smaller concentration applied to the sand columns (approx.  $1 \times 10^4$  genome copies / mL) relative to the other viruses (approx.  $1 \times 10^6 - 10^7$  genome / mL), pathogen inactivation, predation or permanent attachment to the coral sand. The methods employed to extract the microorganisms from the coral sands might not have been adequate to detect the microorganisms, especially norovirus, from the coral sand.

It was not explored but if repeated flushing, significant changes in ionic strength (i.e. multiple rainfall events) or pH had occurred during the experiments then the mobilisation of the viruses might have occurred at the lower levels of each column (220 - 400 mm).

## 6. Final Discussion

An unsaturated laboratory column study was conducted on coral sand sourced from Kiribati to investigate its effective properties and performance as a porous microbial filter medium for potential use in an engineered septic tank effluent drainage field. The investigation:

1. Evaluated the effective pathogen removal rates on: i) clean coral sand, ii) coral sand conditioned with domestic effluent
2. Studied the impact of a flush of groundwater and a high rainfall event on the removal properties of coral sand
3. Examined the packing properties of coral sand and the risk of preferential flow
4. Examined the microbial attenuation properties of coral

Two main conditions were investigated during the experiments on the coral sand. One set of experiments focused on clean coral sand while the other set focused on coral sand that had been conditioned with domestic effluent.

The clean coral sand experiments found that none of the microorganisms applied to the columns were detected in the drainage from any of the columns. This result is likely to be due to the microorganisms being physically retained within the coral sand due to unsaturated conditions. This result highlights the benefit and importance of managing a coral sand effluent disposal field carefully to maintain unsaturated conditions to maximise the opportunities for microbial removal. During the groundwater application experiment the enumerated microorganisms (*E. coli* J6-2, *E. faecalis* and MS2 phage) were too numerous to count. This results highlights the vulnerability of a septic tank effluent drainage field not only to fluctuations in groundwater but also to the application of large volumes of groundwater which would most likely come from households that use groundwater for household washing and flushing toilets that then enter household septic tanks. Given these results a raised or mounded coral sand effluent disposal field would be

recommended to not only increase the distance between the application point within the disposal field bed in relation to groundwater level but to also maximise the available treatment thickness of coral sand within a disposal bed.

The conditioned coral sand experiments were expected to achieve different results due to the conditioning of the columns with a source of DOC in the form of domestic effluent. The results showed that the largest organisms *E. coli* J6-2 and *E. faecalis* were the only microorganisms to be enumerated in the column drainage while the indicator and pathogenic viruses were not detected. The observed mobility of *E. coli* J6-2 and *E. faecalis* was most likely due to their size and the conditioning of the coral sand with DOC which most likely affected the available adsorption sites. During the subsequent experiments on the conditioned coral sand the rainfall experiment observed similar results to the conditioned coral sand tracer experiment in that *E. coli* J6-2 and *E. faecalis* were the only microorganisms to be enumerated in the column drainage. The following dye tracer experiment that used the same rainwater showed the mobilisation of all microorganisms apart from rotavirus and norovirus. The dye tracer experiment also showed that there appeared to be no risk of preferential flow which would have been observed by mottling. These results highlight the potential benefit of designing a graded effluent disposal bed which layers the coral sand from smaller to larger clasts in order to attempt to physically strain the larger microorganisms such as *E. coli* J6-2 and *E. faecalis*.

## 6.1. Conclusions

The following conclusions can be drawn from the coral sand microbial removal studies:

1. Bikenibeu beach coral sand is effective at attenuating microorganisms most likely through the removal mechanism of adsorption, especially for indicator and pathogenic viruses (MS2 phage, adenovirus, echovirus and rotavirus), but less effective for bacterial indicators (*E. coli* J6-2 and *E. faecalis*). No attenuation conclusions could be made for norovirus
2. A coral sand effluent disposal field needs to avoid interaction with sources of groundwater and rainwater to minimise the mobilisation of microorganisms into the valuable groundwater resources of South Tarawa.



3. Disposal field needs to be designed in such a way to mitigate the mobilisation of pathogens due to heavy rainfall events (i.e. mounded disposal field)
4. *E. coli* J6-2 and MS2 phage would be suitable indicator organisms for a field scale experiment (i.e. *E. coli* J6-2 in this research was conservative while MS2 phage was a good surrogate for viral pathogens)
5. Low flow effluent application is most likely to be effective in maximising microbial removal
6. Formation and maintaining a *Schmutzdecke* layer is likely to be most important for microbial removal within a coral sand domestic effluent disposal field.

The findings of this research mark an early step towards providing a standardised design and alternative domestic effluent treatment technology for Kiribati, which utilises locally available coral sand.

## 6.2. Future research needs

From the results of this research the following future research needs have been identified:

1. Assessing the benefit of conditioning the coral sands for a longer period of time to form a mature *Schmutzdecke* and the determining the impact of DOC application on the microbial removal effectiveness of coral sand,
2. Determining the potential impact of various effluent application rates on the microbial removal within coral sand,
3. Determining the potential impact of various porosities and bulk densities on the microbial removal within coral sand without reducing its effectiveness (i.e. clogging from fine coral sand particles)
4. Conducting batch tests to determine if adsorption is a significant microbial removal mechanism within coral sand
5. Long term field scale experiment to determine the practical implications of the design and performance of coral sands as a porous microbial removal medium for domestic effluent disposal fields. Equally, a direct study of conventional effluent drainage field practices (i.e. undisturbed natural soil conditions) should be conducted to enable a comparative study to be made.

6. Future practical assessments should also incorporate: the effects of king tide events (i.e. saline water inundation); fluctuations in groundwater; effects of prolonged heavy rainfall; and examine microbial transport under saturated conditions.
7. The addition of a source of organic carbon (i.e. coconut husk) should also be incorporated into a coral sand disposal field design to provide some level of nutrient treatment, especially for nitrogen and phosphorus.
8. Setting minimum design standards for on-site wastewater treatment systems which utilise coral sand effluent disposal fields on South Tarawa, Kiribati.

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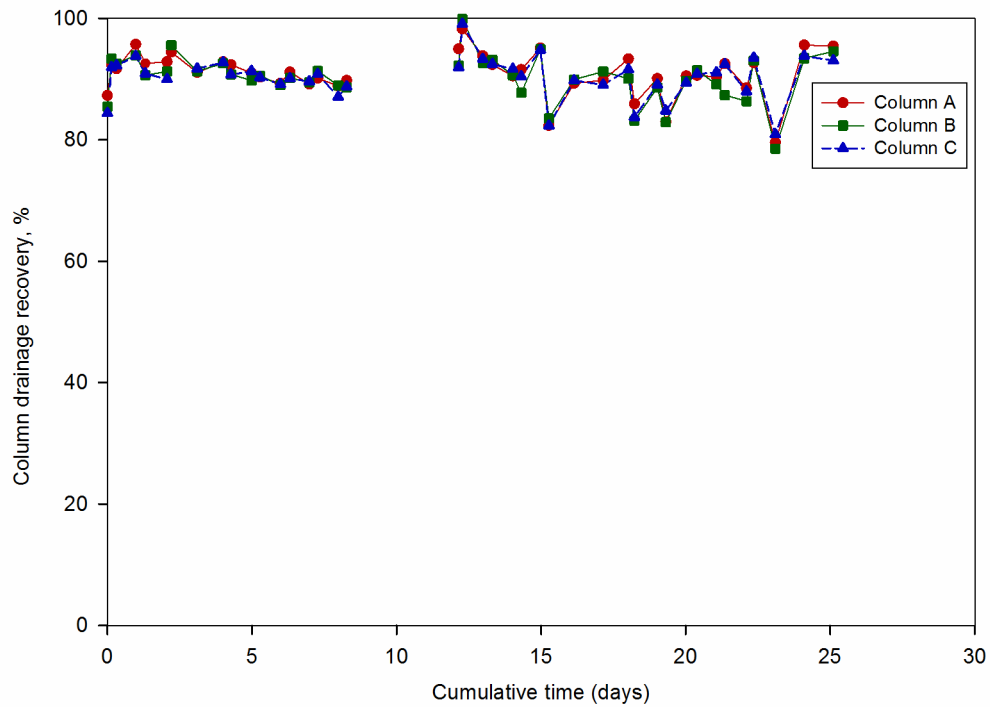
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# A. Additional data

## A.1. Column drainage recovery

The following drainage recovery percentage results were recorded during the coral sand column conditioning with domestic effluent for 27 days.



**Figure A.1.:** Column drainage recovery percentage (%) during the coral sand column conditioning with domestic effluent for 27 days.